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Drinking Water Criteria Document on Chromium

Life Systems, Inc., Cleveland, OH

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16. ABSTRACT

This document deals with the drinking water health effects of Chromium through the review of several studies. These studies include animal and humans. Physical and chemical properties are discussed. Carcinogenicity of the compound is reviewed and evaluated through various studies. Any existing guidelines and standards are presented.

17. KEY WORDS AND DOCUMENT ANALYSIS

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FOREWORD

Section 1412 (b)(3)(A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish Maximum Contaminant Level Goals and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgment of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The Maximum Contaminant Level Goal is non-enforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the Maximum Contaminant Level Goal include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the Maximum Contaminant Level Goals. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity were evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document was comprehensive, only the reports considered most pertinent in the derivation of the Maximum Contaminant Level Goal are cited in the document. The comprehensive literature search in support of this document includes information published up to October, 1983; however, more recent information may have been added during the review and regulatory process. Specifically, the Criteria Document has been updated in response to comments received following the publication of the intent to regulate in 1983 and proposed Maximum Containment Level Goal values in 1985 and 1989. Significant research data were added to the Criteria Document during these revisions.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (One-day, Ten-day and Longer-term, approximately a 7-year exposure) are included in this document. These values are not used in establishing the Maximum Contaminant Level Goal, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

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I. SUMMARY

Chromium exists in the environment in three principal states: elemental (Cr), trivalent (Cr(III)), and hexavalent (Cr(VI)). In general, Cr(III) compounds are relatively insoluble in water and exhibit a low migratory potential in soil, whereas Cr(VI) compounds are more soluble and have a greater potential to migrate. Both states Cr(III) and Cr(VI), appear to be interconvertible under natural water conditions. Specifically, Cr(VI) is reduced to Cr(III) by Fe(II), dissolved sulfides, and certain organic compounds with sulfhydryl groups, whereas Cr(III) is oxidized to Cr(VI) by manganese dioxide, chlorine and by O_2 (at a slow rate).

In humans and experimental animals, gastrointestinal absorption of inorganic salts of Cr(III) is low (from 0.5% to 3%). However, Cr(VI) and organic complexes of Cr(III) are more readily absorbed (approximately 2% to 10% for Cr(VI) and 10% to 25% for organic complexes of Cr(III)). Flameless atomic absorption measurements of total chromium levels in human hair suggest that gastrointestinal absorption of total chromium in infants and young children generally exceeds that found in adults. Precise levels for chromium absorption by infants have not been reported. The distribution of chromium in tissues varies considerably depending on the oxidation state and the chemical form of chromium administered. In mice and humans, the levels of chromium in tissues generally decline with increasing age. Polynomial regression analysis has indicated that there are at least three kinetic compartments in which chromium is retained in rats, but the identity of these pools in the body is not yet clear. The major excretory route for chromium, accounting for 80% or more of chromium excretion, is the urinary system, although the chemical form in which chromium is excreted is not yet known.

The principal routes of chromium exposure in humans are drinking water, food and air. Water concentrations of chromium are generally less than 5 µg/L. However, approximately 215,000 people in the U.S. on public water systems are supplied with water containing more than 50 µg/L. Chromium intake from the diet ranges from 5 to 500 µg/day and averages 100 µg/day. Intake of chromium from the air ranges from 0.03 to 0.3 µg/day and averages 0.1 µg/day. Thus, people drinking high-chromium water (50 µg/L) receive about one half of their chromium intake from food and one half from drinking water, and those with average-chromium water (5 µg/L) receive about 91% from food and 9% from water.

Animal studies have demonstrated that small doses of Cr(III) (2.5 to 5.0 µg/kg) are essential for good health. In rats, chromium enhances glucose uptake by fat tissue, decreases circulating cholesterol levels without affecting the levels of aortic lipids, decreases aortic plaques and increases the life span of the animal.

There do not appear to be any detrimental effects from long-term ingestion of low levels of chromium in drinking water. In rats the maximum nontoxic level of potassium chromate (K_2CrO_4) was 500 ppm (approximately 134 mg Cr(VI)/L or 80 mg Cr(VI)/kg/day). Sprague-Dawley rats administered water containing 25 mg/L chromium chloride (approximately 2.1 mg Cr(III)/kg/day) or potassium chromate (approximately 2.1 mg Cr(VI)/kg/day) for one year exhibited no pathological effects. Female dogs administered 0.45 to 11.2 mg/L of K_2CrO_4 (approximately 0.012 to 0.30 mg Cr(VI)/kg/day) in their drinking water for four years showed no abnormalities in physical condition, food consumption or growth rate.

However, parenteral administration of higher doses of chromium may produce toxic effects. In subchronic experiments using male rabbits, intraperitoneal injection of either Cr(III) or Cr(VI) at 2 mg/kg/day for three to six weeks produced congestion and tissue degeneration in the brain, kidney and myocardium. In another study using rabbits, intraperitoneal injection of Cr(III) and Cr(VI) compounds (approximately 2 mg/kg/day) caused degeneration of the seminiferous epithelium of the testis and disappearance of spermatocytes.

Acute toxicity studies indicate that the LD_{50} for Cr(III) administered intravenously to rats is 10 mg/kg. Continuous inhalation exposure of male Sprague-Dawley rats to sodium chromate (Cr(VI)) at 0.173 $mmol/m^3$ in air causes extensive damage to the tracheal epithelium and submucosal glands within 24 hours. A total dose of 1.25 mg of potassium dichromate (Cr(VI)) injected into the renal artery of mongrel dogs produces preferential damage to the first third of the proximal convoluted tubule within 72 hours. Intraperitoneal injection of massive doses of sodium chromate (3.5 to 7 mg Cr(VI)/kg) to male rats has also produced damage in the proximal convoluted tubule.

Limited evidence suggests that Cr(III) is not teratogenic. Injection of Cr(VI) into chicken eggs did cause teratogenic and lethal effects in the embryos, but this is not strong evidence that Cr(VI) ingested orally by the hen would be teratogenic.

Many mammalian and microbial mutagenicity studies indicate that Cr(VI) compounds are mutagenic. However, other studies suggest that exogenously administered Cr(III) is not mutagenic. Compounds containing Cr(VI) (sodium dichromate, chromic acid, calcium chromate and potassium dichromate) consistently increased His^+ revertants in Salmonella typhimurium at concentrations of 10 to 200 μg/plate, whereas Cr(III) compounds (chromium potassium sulfate and chromium chloride) did not. Both chromium chloride (Cr(III), 0.4 μg/mL) and potassium dichromate (Cr(VI), 0.1 μg/mL), when added separately to tertiary fetal mouse cell cultures, produced chromosomal aberrations.

The possible carcinogenicity of chromium compounds has been widely investigated in experimental animals. Tumors are not induced in animals exposed to either Cr(VI) or Cr(III) by inhalation or ingestion. Administration of Cr(VI) compounds by injection has produced localized sarcomas at the injection site, but many "noncarcinogenic" substances induce injection-site sarcomas.

Acute chromium poisoning is rare in humans. Ingestion of 0.5 to 1.5 gm of potassium dichromate (Cr(VI)) can be fatal, causing liver and kidney damage as well as thrombocytopenia and internal hemorrhage. Chronic ingestion of water containing 1 mg/L of Cr(VI) over a three-year period did not produce any adverse health effects in a Long Island family drinking from a private well. It should be noted that the degree of medical follow-up of this family, as reported in the literature, was limited to physical examinations.

Subchronic and chronic exposure to Cr(VI) in the form of chromic acid can cause contact dermatitis and ulceration of the skin in humans. Chronic inhalation of dust or air containing Cr(VI) or Cr(III) can cause respiratory effects including perforated or ulcerated nasal septa and decreased spirometric values. Recent studies have suggested that inhalation of Cr(III) compounds does not pose a significant carcinogenic risk to humans. However, an association between prolonged inhalation of Cr(VI) compounds and the development of cancer of the respiratory tract has been suggested by epidemiological studies.

Compounds of Cr(VI) are generally more toxic than Cr(III) compounds because they are more soluble, readily traverse biological membranes and have strong oxidizing properties. After traversing cell membranes, Cr(VI) is thought to be reduced intracellularly to Cr(III), which forms octahedral complexes with cellular macromolecules including proteins and nucleic acids. By interacting with DNA, Cr(III) may influence base-pairing and the conformation of DNA, thereby causing somatic mutations. This interaction with nucleic acids may be responsible for the mutagenic and putative carcinogenic properties of chromium. Chromium may also bind to intracellular enzymes and exert its toxic effects by interfering with their function. In this regard Cr(III) as the soluble nitrate salt appears to be more active than Cr(VI) (as potassium dichromate).

An estimated adequate and safe intake for chromium of 50 to 200 µg/day for human adults has been established. This range is based on the absence of signs of chromium deficiency in major segments of the United States population consuming an average of 0.06 mg (60 µg) of chromium per day. Estimated daily adequate and safe intakes for younger age groups are derived by extrapolation on the basis of expected food intake.

No suitable data for calculating a Cr(VI) One-day Health Advisory (HA) were identified. Therefore, the Ten-day HA is taken as a conservative estimate of the One-day HA. Using a No-Observed-Adverse-Effect Level (NOAEL) of 14.4 mg/kg/day for Cr(VI) from a 60-day study with rats, a Ten-day HA of 1.4 mg/L was calculated for a 10-kg child. A one-year drinking water study in rats that derived a NOAEL of 2.41 mg/kg/day for Cr(VI) was used to calculate Longer-term HAs of 0.24 mg/L and 0.84 mg/L for a 10-kg child and 70-kg adult, respectively. There were no suitable data for calculating One-day, Ten-day or Longer-term HA values for Cr(III). Further, the absence of dose-response data prevented the derivation of a Reference Dose (RfD) and Drinking Water Equivalent Level (DWEL) for Cr(III). Thus, the RfD and DWEL were derived based on data for Cr(VI), and are considered to be conservative estimates of the values for Cr(total). The NOAEL of 2.41 mg/kg/day from the one-year study on rats that was used to calculate the Longer-term HA was also used to calculate an RfD of 0.0048 mg/kg/day and a DWEL of 0.17 mg/L. Although the same data were used, the DWEL differs from the Longer-term HA because an additional uncertainty factor of five was employed in the DWEL calculation to compensate for use of data from a less than lifetime study.

Insufficient data are available to perform a quantitative risk analysis for cancer following oral exposure to chromium. A tentative link between cancer and chromium has been made based on epidemiological studies in industry where there is chronic inhalation exposure to Cr(VI). However, no other evidence of carcinogenicity is available, and since occupational exposure levels of chromium exceed those of nonoccupational sources (food, water and air) by several orders of magnitude, the risk of cancer following ingestion of chromium in drinking water at 100 μg/L appears to be negligible.

Recommended or established standards for chromium in the United States include: The ambient water quality criterion of 50 μg/L for Cr(VI) and 170 mg/L for Cr(III) (USEPA 1980); 1 $\mu g/m^3$ for carcinogenic forms of Cr(VI) in workplace air (NIOSH 1975); and 25 $\mu g/m^3$ TWA or 50 $\mu g/m^3$ ceiling for non-carcinogenic forms of Cr(VI) in workplace air (NIOSH 1975).

II. PHYSICAL AND CHEMICAL PROPERTIES

A. Physical and Chemical Properties

Elemental chromium is a blue-white, refractory metal that exists in the earth's crust in relative abundance (Westbrook 1979). Some of its properties are listed in Table II-1.

Chromium can exhibit oxidation states from -2 to +6. In the natural environment, where oxygen is present, chromium exists in three principal states: elemental (Cr), trivalent (Cr(III)) and hexavalent (Cr(VI)) (Westbrook 1979). Thermodynamic data indicate that the Cr(III) and Cr(VI) species are stable in pure water (Latimer 1952). In general, Cr(VI) salts are more soluble than those of Cr(III) (Weast 1971), whereas Cr is relatively insoluble (e.g., 0.5 µg Cr/L between pH 8.0 and 9.5) (Hem 1970). The physical constants for some chromium salts are shown in Table II-2.

In solution, Cr(III) has a tendency to coordinate with electron-pair donors, with chelating agents and with strong Lewis bases (Hartford 1979). Cr(III) has a coordination number of six and the resulting compounds or complexes are octahedral and relatively insoluble in water (Cotton and Wilkinson 1972).

For example, with ligands such as 2,4-diketones, which combine the functions of a monoprotic acid and bidentate ligand, Cr(III) forms neutral

Table II-1 Properties of Elemental Chromium

Atomic Number	24
Atomic Weight	51.996
Density	7.14 g/cm^3
Melting Point	1,900 C
Boiling Point	2,642 C

Adapted from Windholz (1976).

Table II-2 Physical Constants of Some Chromium Salts

Salt	Boiling point (°C)	Melting point (°C)	Solubility in cold water (g/100 mL)	Density (g/cm^3)
Cr(III)				
$Cr(C_2H_3O_2)_3 \cdot H_2O$			slightly soluble[a]	
$CrCl_3$	subl 1,300	ca 1,150	insoluble	2.76^{15}
$CrCl_3 \cdot 6H_2O$		83	58.5^{25}	1.76
$Cr(NO_3)_3 \cdot 9H_2O$	d at 100[b]	60	soluble	
$Cr_2(SO_4)_3 \cdot 18H_2O$		-12 H_2O, 100	120.0	1.7^{22}
Cr(VI)				
$(NH_4)_2CrO_4$		d at 180	40.5^{30}	1.91^{12}
CrO_3	d	196	61.7^{0}	2.70
K_2CrO_4		968.3	62.9^{20}	2.732^{18}
$Na_2Cr_2O_7 \cdot 2H_2O$	d at 400 (anhydr)	-2 H_2O, 100	238.0^{0}	2.52^{13}

[a] No concentrations given.
[b] d = decomposes.

Adapted from Weast (1980).

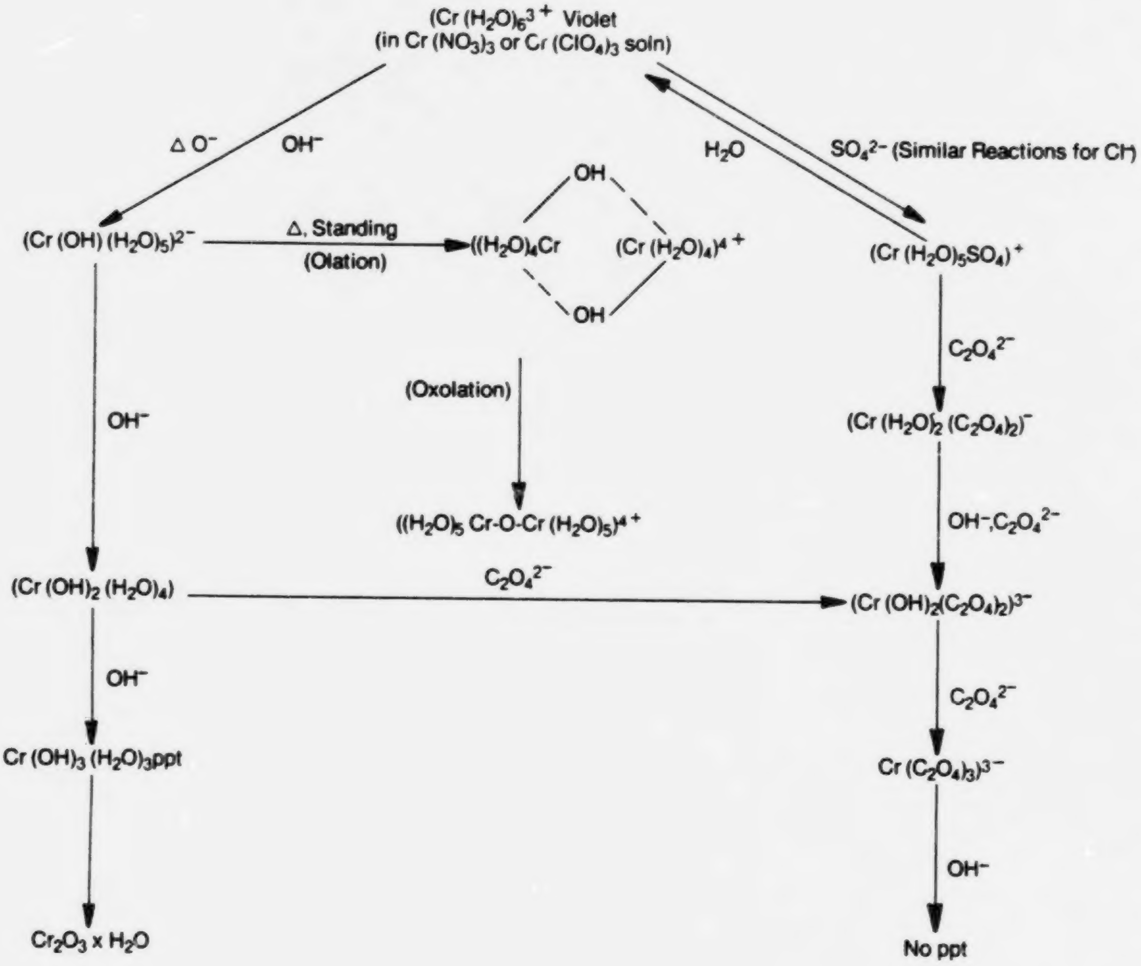
complexes such as chromic tri(acetylacetonate), a non-electrolyte that is nearly insoluble in water (Hartford 1979).

Complexes of chromium chloride ($CrCl_3$) with Lewis-base organic solvents can be conveniently prepared by refluxing $CrCl_3$ in the presence of a small amount of zinc in a Soxhlet extractor (Hartford 1979).

When hydroxyl ions are added to Cr(III) in quantities insufficient for precipitation, basic ions are formed, which then polymerize and condense, with the formation of ol and oxo bridges. Such ions are of the appropriate size to cross-link protein fibers and play an important part in the chemistry of tanning. Some typical reactions involving OH^-, a weakly coordinated ion such as SO_4^{2-}, and a strongly coordinated ion such as $Cr_2O_4^{2-}$ are shown in Figure II-1 (Hartford 1979).

Cr(III) compounds can be reduced to Cr(II) derivatives by hypophosphites, electrolysis, or active metals such as zinc, magnesium and aluminum in acid solution. Cr(III) can also be oxidized slowly to Cr(VI), the rate increasing with increasing pH and temperature (Hartford 1979). In basic solutions, Cr(III) is readily oxidized to Cr(VI) by hypochlorite, hypobromite and peroxide and by oxygen under pressure and high temperature (Hartford 1979).

Cr(III) and Cr(VI) appear to be readily interconvertible under natural water conditions (Schroeder and Lee 1975). Specifically, Cr(VI) is reduced by Fe(II), dissolved sulfides and certain organic compounds with sulfhydryl


(Cr (H2O)6)3+ Violet
(in Cr (NO3)3 or Cr (ClO4)3 soln)
Δ O⁻
OH⁻
H2O
SO4 2- (Similar Reactions for Cl⁻)
(Cr (OH) (H2O)5)2-
Δ, Standing
(Olation)
((H2O)4Cr
OH
OH
(Cr (H2O)4)4+
(Cr (H2O)5SO4)+
(Oxolation)
OH⁻
C2O4 2-
(Cr (H2O)2 (C2O4)2)⁻
((H2O)5 Cr-O-Cr (H2O)5)4+
OH⁻, C2O4 2-
(Cr (OH)2 (H2O)4)
C2O4 2-
(Cr (OH)2(C2O4)2)3-
OH⁻
C2O4 2-
Cr (OH)3 (H2O)3 ppt
Cr (C2O4)3)3-
OH⁻
Cr2O3 x H2O
No ppt

Adapted from Hartford (1979).

Figure II-1 Some Typical Ion Relationships of Cr(III)

groups, whereas Cr(III) is oxidized to Cr(VI) by a large excess of manganese dioxide (25 mg MnO_2/L), free residual chlorine (2 mg/L) and at a slow rate (1% per week), by O_2 under conditions approximating those in natural waters (Schroeder and Lee 1975; Ulmer 1986).

In a playa-type, sedimentary environment, Robertson (1975) found that at pH 9.0 and above, oxygenated ground water chemically oxidized Cr(III) to Cr(VI) in the silt-clay fraction of a closed basin. Thus, it would appear that the oxidation of Cr(III) to Cr(VI) occurs readily under alkaline and aerobic conditions. In most landfill or impoundment disposal situations, acidic and anaerobic conditions normally prevail (Stanforth et al. 1979); hence, the oxidation of Cr(III) to Cr(VI) is unlikely to occur (Hartford 1979; Bartlett and Kimble 1976a).

Cr(III) can be strongly adsorbed by clays and by organic materials at pH 4 and above, with the adsorption increasing with increasing pH (Bartlett and Kimble 1976a; Griffin et al. 1977). In contrast, Cr(VI) compounds are generally water soluble and have a relatively higher migratory potential in soil (Bartlett and Kimble 1976b; Griffin et al. 1977). However, Cr(VI) salts are readily reduced to less soluble Cr(III) forms by soil organic matter (Bartlett and Kimble 1976b). Landfill disposal of Cr(VI) wastes would therefore probably pose a pollution hazard only when the concentration of Cr(VI) exceeded both the adsorbing and reducing capacities of the soil (Bartlett and Kimble 1976b).

Cr(VI) linked with oxygen is a strong oxidizing agent (Cotton and Wilkinson 1972; Mertz 1969). In solution, Cr(VI) exists as a part of a complex anion rather than as a cation and does not, therefore, readily precipitate at alkaline pH. Important anions are the hydrochromate, chromate, and dichromate. The latter two are relatively stable in groundwater environments (Hem 1970). The proportion of Cr(VI) present in each of these forms is pH-dependent (Trama and Benoit 1960). In natural waters the proportion of dichromate ions is low. In acid environments the hydrochromate ion predominates (Trama and Benoit 1960); at alkaline pH, however, chromate ion is the major form (Trama and Benoit 1960). In rather strongly reducing environments in which the pH is below 8, the predominant form is the complex Cr(III) ion bearing an associated hydroxyl group ($CrOH^{2+}$) (Hem 1970).

B. Manufacturing and Uses

The most important commercial chromium ore is chromite, which contains from 22% to 38% chromium (Morning 1980). Information on the annual U.S. production of chromium is not available, but a total of 327,000 metric tons of chromium were consumed in the U.S. in 1976 (Morning 1980; Hartford 1979). Hexavalent chromium compounds are widely used in industry for chrome alloy and chromium metal production, for metal finishing and corrosion control (Love 1947), and as mordants in the textile industry (Iler 1954). Chromium salts are also used as anticorrosive agents in cooling waters, in the leather tanning industry, in the manufacture of catalysts, in pigments and primer paints and in fungicides and wood preservatives (Hartford 1979).

C. Summary

Chromium exists predominantly as elemental Cr, Cr(III) and Cr(VI) in the natural environment. Cr(III) and Cr(VI) are interconvertible under the proper conditions. Oxidation of Cr(III) to Cr(VI) can occur in basic environments with adequate oxygenation, whereas Cr(VI) is readily reduced to Cr(III) by organic matter. Most Cr(III) compounds are relatively insoluble in water and exhibit a low migratory potential in soil; in contrast, Cr(VI) compounds are more soluble and have a higher potential for migration. In 1976 there were 327,000 metric tons of chromium used in the United States, in products as diverse as car bumpers and wood preservatives. Sensitive methods recently developed allow rapid analysis of Cr(VI) in a mixture with Cr(III).

III. TOXICOKINETICS

A. Absorption

The levels of gastrointestinal absorption of Cr(III) in the form of inorganic salts have been reported to be low by numerous investigators. Estimates of gastrointestinal absorption of Cr(III) in humans can be obtained from the excretion data of Donaldson and Barreras (1966). After oral administration as $^{51}CrCl_3$ or $Na_2{}^{51}CrO_4$, chromium was almost completely recovered in feces. When placed into the duodenum, Cr(VI) as chromate was absorbed to a significant extent (Table III-1). The dosage of each salt was 20 ng (2.9×10^{-7} mg/kg based on a 70-kg weight for reference man (ICRP 1975)) and was administered to fasting subjects in water. The volume of solution administered was not reported. Fecal recoveries of the oral and duodenal doses of Cr(III) administered were 99.6% and 93.7%, respectively. Thus, low levels of gastrointestinal absorption of Cr(III) occur following administration of $^{51}CrCl_3$ by either ingestion or injection into the duodenum.

Visek et al. (1953) have reported less than 0.5% intestinal absorption of a single dose of $^{51}CrCl_3$ (Cr(III)) in rats; the dosage administered was not reported. Mertz et al. (1965) reported 2% to 3% absorption of Cr(III) from total body counting of rats given doses of $^{51}CrCl_3$ ranging from 1.5 to 100 µg/kg by stomach intubation.

Reports by Mertz (1976) and Mertz et al. (1978) suggest markedly greater absorption of Cr(III) in the form of glucose tolerance factor (GTF) (10% to

Table III-1 Urinary and Fecal Excretion of Radioactivity Following Oral and Duodenal Administration of $^{51}CrCl_3$ and $Na_2{}^{51}CrO_4$ to Patients[a]

	% Radioactivity excreted					
	Oral administration			Duodenal administration		
	Feces	Urine	Total excreted	Feces	Urine	Total excreted
$^{51}CrCl_3$ (Cr(III))						
No. of patients	6	6		2	2	
Range	96.8-101.3	0.1-1.2		90.2-97.1	0.2-1.0	
Mean ± S.D.	99.6±1.8	0.5±0.3	100.1	93.7±4.5	0.6±0.5	94.3
$Na_2{}^{51}CrO_4$ (Cr(VI))						
No. of patients	5	6		4	4	
Range	84.6-96.7	0.2-4.4		46.0-82.2	6.6-49.3	
Mean ± S.D.	89.4±2.6	2.1±1.5	91.5	56.5±11.7	10.6±5.6	67.1

[a] A single dose of 20 ng of either $^{51}CrCl_3$ or $Na_2{}^{51}CrO_4$ (2.9×10^{-7} mg/kg based on weight for reference man ICRP 1975)) was administered to each subject.

Adapted from Donaldson and Barreras (1966).

25% of administered dose) than of inorganic Cr(III) salts (0.5%-3.0%) in the rat (Donaldson and Barreras 1966; Visek et al. 1953; Mertz et al. 1965). Glucose tolerance factor is a natural complex of Cr(III) with nicotinic acid and amino acids that is found in brewers yeast (Toepfer et al. 1977; Mertz et al. 1978). Glucose tolerance factor was prepared by extracting brewers yeast with dilute alcohol and was purified by ion-exchange chromatography. The presence of nicotinic acid in GTF was established by the presence of a pyridine structure in mass spectrographs and the observed peak in optical density scans at 262 nm. The ultraviolet spectrum of sublimed material from the GTF preparation also showed absorption typical of nicotinic acid. Amino acid analyses showed the predominant amino acids present to be glycine, glutamic acid, and cysteine (Toepfer et al. 1977).

The gastrointestinal absorption of Cr(VI) has been reported to be somewhat higher than that of Cr(III) in the form of inorganic salts. An estimate of 2.1% absorption of Cr(VI) can be made from the excretion data of Donaldson and Barreras (1966), who administered a single oral dose of 20 ng of $Na_2{}^{51}CrO_4$ (Cr(VI)) (2.9×10^{-7} mg/kg based on 70-kg weight for reference man (ICRP 1975)) in water to fasting subjects. The volume of solution of the oral dose was not stated. However, when 20 ng of $Na_2{}^{51}CrO_4$ was injected slowly through a fine polyethylene tube into the third portion of the duodenum, markedly higher levels of Cr(VI) absorption (10.6%) occurred. The volume of injected solution was not given. Within 24 hours, 10.6% of the administered dose was excreted in the urine. No explanation was given by the authors for the total recovery of only 67.1% of intraduodenally administered dose of Cr(VI). Because of the low recovery of Cr(VI), it is possible that absorption may actually have been greater than the 10.6% reported. However, considerably higher levels of recovery (91.5%) were reported for oral administration of Cr(VI) as $Na_2{}^{51}CrO_4$ by the same investigators, with less chromium being excreted in urine. The significance of this finding is not clear, as urinary excretion can provide only a minimum value for absorption.

According to flameless atomic absorption analysis of total chromium in human hair, it appears that chromium retention in infants exceeds that in adults (Saner and Gurson 1976). The results in Table III-2 indicate a significant increase in the chromium concentrations of hair from newborns and infants up to two months of age (greater than a three-fold increase, $P \leq 0.01$), but no marked change in the concentrations of chromium in the hair of the mothers over the same two-month period. Also, the mean level of total chromium in two-month-old infants (443 ng chromium/g hair) was 1.8 times the mean level reported for the mothers at the end of the two-month period (249 ng chromium/g hair); this marked difference suggests a relatively high absorption and/or retention of chromium in infants and young children. Precise levels for chromium absorption in infants were not found in the available literature.

There were no reliable data available to assess pulmonary absorption of chromium.

Table III-2 Chromium Concentrations in the Hair of Mothers and Their Newborns at Delivery and Two Months After Delivery[a]

Number of cases	Chromium concentrations (ng/g hair)		Chromium concentration of mothers (ng/ hair)	
	Newborn	Infant (2 months)	At delivery	2 months after delivery
1	441	731	417	177
2	244	222	120	144
6	70	667	541	304
9	35	549	298	554
10	129	360	239	309
11	103	451	180	387
16	96	445	76	93
18	142	237	133	155
20	30	325	252	122
Mean ± SEM[b]	143 ±43	443 ±60	251 ±50	249 ±51

[a] Hair chromium concentrations: newborns versus infants (2 months old, $P \leq 0.01$, mothers at delivery versus two months after delivery) no significant difference.

[b] SEM: the standard error of the mean.

Adapted from Saner and Gurson (1976).

B. Distribution

Chromium normally circulates in the plasma primarily in a nondiffusible form. In an *in vitro* study, the distribution of Cr(III) in the plasma protein fractions of rats was shown to be dose-dependent (Hopkins and Schwarz 1964) (Table III-3). At low levels (0 to 1 μg Cr(III)/mL serum), the iron-binding protein siderophilin, complexed most of the Cr(III) present, but at higher levels (10 μg Cr(III)/mL serum) other plasma proteins including alpha 1 and alpha 2 globulin and the albumin fractions, were involved (Hopkins and Schwarz 1964). Blood levels of chromium in 20 normal Japanese subjects were 2.9 ng/mL (Nomiyama et al. 1980).

A small fraction of plasma chromium (9% to 12%) is also present in a more diffusible form which is filtered at the glomerulus and partially reabsorbed in the renal tubule (Collins et al. 1961). The fraction of plasma chromium present in the diffusible form (dialyzable chromium) was determined by equilibrium dialysis in blood from mongrel dogs administered a single intravenous dose ranging from 5 to 1,900 μg/kg of $^{51}CrCl_3$ (Cr(III)). This method assumed that the binding of chromium to plasma proteins was essentially irreversible under the dialysis conditions used, as demonstrated by Gray and Sterling (1950).

Trivalent chromium shows an affinity for transferrin similar to that of iron; evidence suggests that the two metals compete for the same binding site (Gray and Sterling 1950).

Table III-3 Influence of Cr(III) on the Subsequent Distribution of ^{51}Cr Among Rat Serum Proteins *In Vitro*

Cr(III) dose[a] (ug/mL serum)	Percent of total radioactivity measured in serum protein fraction				
	Gamma	Beta	Alpha-2	Alpha-1	Albumim
0	2.0	85	5.9	2.2	4.4
0.1	0.0	87	4.4	2.7	6.1
1	5.6	77	7.2	3.3	6.7
10	5.5	56	18.0	7.2	13.2

[a] The unlabeled amounts of Cr(III) indicated were added 5 minutes before the addition of 0.1 mCi of carrier-free ^{51}Cr.

Adapted from Hopkins and Schwarz (1964).

Siderophilin binds iron, producing an intense color at 470 nm which is proportional to the amount of iron added. When $CrCl_3 \cdot 6H_2O$ was added to serum very little color change was produced, although $CrCl_3 \cdot 6H_2O$ apparently interfered with the siderophilin-iron complex when the protein became saturated with both iron and chromium. This interference may have resulted from chromium binding at the same site on the protein as iron (Hopkins and Schwarz 1964). Although chromium in the trivalent form is transported in the blood, it is unable to cross the erythrocyte membrane (Gray and Sterling 1950).

Metal ligands are important in determining the movement of heavy metals within the body (Collins et al. 1961; Foulkes 1974). It is not surprising, therefore, that the distribution of chromium in the body also critically depends on the presence of specific ligands (Mertz and Roginski 1971). *In vitro*, the presence of ligands, such as amino acids, and some chelating agents, such as EDTA and DL-penicillamine, increases chromium transport (Mertz and Roginski 1971).

The half-life of chromium in the plasma is relatively short. Hopkins (1965) found that the levels of ^{51}Cr(III) decreased to 50% of their original values in the blood of male rats within approximately six hours following intravenous administration of either 0.01 or 0.1 μg Cr(III)/100 g.

Cells tend to accumulate chromium to levels higher than those present in the plasma. Presumably, this results from intracellular trapping of chromium compounds, that penetrate in the hexavalent form, are reduced to the trivalent form, then react with cellular constituents, such as hemoglobin in the case of erythrocytes (Gray and Sterling 1950), or proteins or nucleic acids (Levis et al. 1978b; Tsapakos et al. 1981). Calculations made from the data of Samitz and Katz (1963) indicate that during in vitro incubation of human skin, a mean of 68% of Cr(VI) traversed the biological membrane and was reduced to Cr(III) (see section III. C., Metabolism).

Considerable variability has been observed in the chromium concentrations of different tissues, depending on the chemical state in which the element is administered. Hopkins (1965) reported that following intravenous administration of low tracer doses (0.01 to 0.1 μg chromium/100 g) of ^{51}Cr-labeled chromium chloride (Cr(III)) to male rats, the spleen, kidney, testis and epididymis retained from 104% to 200% of their early levels after four days. The early ^{51}Cr levels per gram of tissue in spleen, kidney, testis and epididymis, measured 15 minutes after administration of 0.1 μg Cr/100 g, represented 65%, 207%, 29% and 28% of the dose administered per g body weight, respectively. Heart, lung and kidney had the highest initial concentrations, but heart and lung rapidly lost radioactivity, whereas the kidneys still retained 104% of their initial concentration after four days. In contrast, the heart, pancreas, lung and brain retained only 10% to 30% of their initial radioactivity after four days. The spleen and kidney continued to concentrate ^{51}Cr throughout the four days, whereas the levels in the testis and epididymis peaked at four and eight hours, respectively (Hopkins 1965).

No data were reported for female rats although the authors stated that little difference was seen between the sexes in either clearance, tissue distribution or excretion of chromium.

The distribution of chromium administered by stomach intubation to two female rats in the form of GTF, however, was entirely different (Table III-4), with liver accumulating the highest concentration, followed by uterus, kidney and bone. Lung, heart, intestines, spleen, pancreas and brain each accumulated about 50% of the concentration found in liver, whereas muscle, ovary and aorta had a considerably lower affinity for chromium (Mertz and Roginski 1971).

The report of MacKenzie et al. (1958) allows a direct comparison of the tissue distribution of Cr(III) and Cr(VI) in male and female rats, as well as an evaluation of the sex differences in tissue distribution. Rats can apparently tolerate up to 25 mg/L of either Cr(III) (as chromic chloride) or Cr(VI) (as potassium chromate) in drinking water for one year (MacKenzie et al. 1958). Based on the average weights of 375 g for a male rat, and 290 g for a female rat and an average daily water intake of 28 ml (Arrington 1972) for these animals, the calculated doses for both Cr(III) and Cr(VI) were 1.87 mg/kg/day and 2.41 mg/kg/day for male and female rats, respectively. The total doses received in one year of administration would be, therefore, 682 mg/kg and 880 mg/kg for the average male and female rat, respectively. The increase over control values of tissue levels of chromium was an average of 12.5 times greater after exposure to 25 mg/L of Cr(VI) than after exposure to the same concentration of Cr(III) (Table III-5). This is consistent with the greater intestinal absorption of the hexavalent form.

Table III-4 Tissue Distribution of ^{51}Cr After Administration of ^{51}Cr-Labeled GTF[a]

	^{51}Cr-labeled GTF concentration[b]	
	Rat No. 1	Rat No. 2
Liver	100	100
Uterus	94	101
Kidney	84	77
Bone	72	62
Lung	61	53
Heart	59	67
Intestines	56	64
Spleen	50	51
Pancreas	49	44
Brain	42	62
Muscle	25	33
Ovary	25	16
Aorta	22	0

[a] Dosage of $^{51}Cr(III)$ was not reported. A pregnant rat received three doses of ^{51}Cr-labeled yeast extract by stomach intubation.

[b] In percent of concentration in liver.

Adapted from Mertz and Roginski (1971).

Table III-5 Chromium Concentrations in Tissues of Rats Given 25 ppm (mg/L) Cr as Potassium Chromate (Cr(VI)) or Chromic Chloride (Cr(III) in Drinking Water for One Year

Chromium concentration (ppm (mg/L))	Kidney (mg/Chromium/ kg wet tissue)[a]		Liver (mg Chromium/ kg wet tissue)		Bone (mg Chromium/ kg wet tissue)		Spleen (mg Chromium/ kg wet tissue)	
	Male	Female	Male	Female	Male	Female	Male	Female
0 Control	0.28±0.04[c]	0.35±0.05	0.05±0.004	0.08±0.012	0.77±0.07	0.78±0.09	0.90±0.08	0.91±0.078
25 Cr(VI)	10.47±0.97	13.79±0.14	3.2±0.31	8.12±0.74	4.07±0.29	8.71±1.38	23.53±1.7	52.85±3.9
25 Cr(III)	1.71±0.29	2.07±0.31	0.35±0.045	0.53±0.017	1.03±0.2	1.23±0.073	3.22±0.45	3.88±0.17

[a] mg Chromium/g wet tissue values reported in MacKenzie et al. (1958) were converted to mg/kg.
[b] ± values are standard errors.

Adapted from MacKenzie et al. (1958).

Spleen was shown to have the highest concentration of chromium in all groups tested including the control group, whereas liver had the lowest. The concentrations of chromium found in the tissues of female rats were consistently higher than those measured in male rats. Tissue concentrations in female rats were greater than those in male rats by factors of 1.3 to 2.5 after administration of Cr(VI) and 1.2 to 1.5 after administration of Cr(III).

In mice, a striking diminution in tissue uptake of Cr(VI) (as sodium chromate) has been observed with increasing age (Vittorio et al. 1962). Following intraperitoneal administration of chromate salts into older mice, the concentrations in liver, stomach, epididymal fat pad, thymus, kidney and testis were markedly lower than those observed in younger animals (Table III-6). The testes and liver were the most affected, whereas incorporation into intestines and lungs appeared not to be influenced by age. These observations may offer one explanation for the declining tissue levels of chromium detected in older people in the U.S. (Schroeder et al. 1962) (Table III-7). For example, total chromium levels in kidney declined from a mean value of 51.8 μg/g tissue ash at age 0 to 45 days to 10.0 μg/g tissue ash at 80 years or more. A marked decrease in total chromium levels in the heart was also reported as chromium levels declined from 82.4 μg/g tissue ash at 0 to 45 days of age to 2.9 μg/g tissue ash at 70 to 80 years and 0 μg/g tissue ash at 80 years or more (Schroeder et al. 1962). It is not clear whether this general decrease in chromium levels is due to dietary deficiencies, decreased uptake by the tissues or release of chromium from the tissues.

Table III-6 The Effect of Age on ^{51}Cr Distribution in Mouse Organs

Ave. age (weeks)	Liver (cpm/mg)	Stomach (cpm/mg)	Intestine (cpm/mg)	Epididymal fat pad (cpm/mg)	Thymus (cpm/mg)	Lung (cpm/mg)	Spleen (cpm/mg)	Kidney (cpm/mg)	Testis (cpm/mg)
7	165.5±25.1[a]	46.2±6.2	45.6±5.36	76.0±7.2	27.3±5.3	29.8±4.5	60.1±36.3	131.8±3.8	47.7±28.8
	(100)[b]	(27.9)	(27.6)	(45.9)	(16.5)	(18.0)	(36.3)	(79.6)	(28.8)
8	156.7±14.2	55.4±5.0	51.3±6.1	59.8±6.9	28.3±4.7	30.9±5.3	100.5±9.2	106.7±4.5	31.3±4.0
	(100)	(35.4)	(32.7)	(38.2)	(18.1)	(19.7)	(64.1)	(68.1)	(20.0)
9	137.5±12.5	44.5±6.9	49.4±6.4	64.0±5.8	27.8±5.7	27.5±4.9	87.2±6.7	108.9±4.9	36.3±4.1
	(100)	(32.4)	(35.9)	(46.5)	(20.2)	(20.0)	(63.4)	(79.2)	(26.4)
12	82.8±11.7	41.4±5.3	48.7±5.3	45.8±6.1	17.6±4.1	28.7±5.0	52.3±5.6	109.7±4.1	24.9±3.1
	(100)	(41.4)	(38.8)	(55.3)	(31.3)	(34.7)	(63.2)	(132.5)	(30.1)

[a] Mice were sacrificed 24 hours after intraperitoneal administration of 25 µCi of sodium radiochromate. Each value, in counts per minute per mg tissue, represents the mean of six mice ± the standard error.
[b] Values in parentheses represent organ concentration as a percentage of liver concentration for that age group.

Adapted from Vittorio et al. (1962).

Table III-7 Levels of Total Chromium in Human Tissue by Age, United States Values[a]

	0-45 days	45 days-10 yr	10-20 yr	20-30 yr	30-40 yr	40-50 yr	50-60 yr	60-70 yr	70-80 yr	80+ yr
Kidney	5.18[b](21)[c]	57.5(25)	3.7(14)	2.3(18)	2.1(38)	3.9(23)	3.0(20)	2.4(11)	2.0(5)	10.0(1)
Liver	17.9(28)	16.6(25)	4.6(14)	2.9(18)	1.8(39)	3.1(23)	2.5(21)	1.3(11)	1.1(5)	1.3(2)
Lung	85.2(12)	10.1(6)	6.8(12)	8.2(18)	15.6(38)	31.8(22)	24.2(21)	21.0(10)	18.0(5)	1.1(1)
Aorta	19.5(4)	7.0(1)	7.0(7)	4.2(11)	9.1(27)	4.9(22)	4.2(15)	4.4(5)	2.6(3)	0(1)
Heart	82.4(9)	1.7(6)	2.1(10)	3.3(18)	3.8(38)	6.0(23)	3.7(20)	3.0(10)	2.9(5)	0(1)
Pancreas	NA[d]	2.6(2)	3.4(12)	2.5(17)	6.5(39)	2.5(21)	3.6(16)	3.7(10)	2.9(4)	0.9(1)
Spleen	27.0(7)	4.1(5)	1.6(12)	1.1(18)	1.7(37)	1.5(22)	1.8(20)	3.0(9)	1.9(4)	0.3(1)
Testis	NA	NA	2.1(5)	1.8(8)	3.1(20)	3.2(12)	2.3(11)	3.6(10)	1.1(5)	1.0(1)

[a] Sex not specified.
[b] Values reported as average µg of total chromium/g tissue ash.
[c] Numbers in parentheses represent number of samples.
[d] NA, no samples available.

Adapted from Schroeder et al. (1962).

Inhalation can lead to an increase in the urinary excretion of chromium. However, it is not clear to what extent the chromium added to systemic pools is absorbed directly from the lungs or from the intestines following pulmonary clearance of chromium-containing particles (Baetjer et al. 1959a). Pulmonary chromium does not appear to be in full equilibrium with other chromium pools in the body. This conclusion is based on the chromium content in the lungs, which unlike that of the rest of the body, may actually increase with age (Schroeder et al. 1962) (see Table III-7). Prolonged pulmonary retention of inhaled chromium is also reflected by the fact that the pulmonary concentration of chromium usually exceeds that of other organs. The relatively slow clearance of chromium from the lungs has also been noted by Baetjer et al. (1959a), sixty days after intratracheal administration of 200 µg of chromium chloride ($CrCl_3$) (Cr(III)) into guinea pigs, they observed that 12% of the dose still remained in the lungs.

The placental transport of chromium (Cr(III)) in pregnant rats apparently depends on the chemical form administered. Inorganic Cr(III) administered as $^{51}CrCl_3$ (chromium chloride) intravenously or by stomach intubation does not appreciably cross the placental barrier in rats (Mertz et al. 1969). However, Cr(III) administered by stomach intubation to pregnant rats in the form of GTF (obtained from yeast) is readily recovered from the fetus (Mertz and Roginski 1971) (Table III-8). Table III-8 shows that not only is ^{51}Cr readily recoverable from fetal tissues after administration of ^{51}Cr-labeled yeast extract, but the amount of radioactivity was higher in fetal tissues than in

Table III-8 ^{51}Cr Concentrations in Maternal and Newborn Tissues[a]

Organ	Subject	^{51}Cr concentration (cpm/g tissue)
Liver	Maternal	48.0
	Fetal	187.0
Heart	Maternal	29.7
	Fetal	45.8
Kidney	Maternal	39.0
	Fetal	44.0

[a] A pregnant rat received three doses of a ^{51}Cr-labeled yeast extract (Cr(III)) by stomach tube. Dosages given were not reported or obtainable from the authors. Radioactivity, reported in counts per minute per gram of tissue, was determined in pooled organs from newborn litter and in corresponding tissues from the mother.

Adapted from Mertz and Roginski (1971).

the corresponding maternal tissues; the level in fetal liver is particularly elevated compared to that of the maternal tissue (3.9 times higher). These results suggest that Cr(III) is much more readily transported across the placental barrier in the rat in the form of GTF than in the form of $^{51}CrCl_3$.

C. Metabolism

The metabolism of chromium in mammalian species is not well understood and is complicated by the presence of the two oxidation states, Cr(VI) and Cr(III). Cr(VI) is reduced to Cr(III), which then reacts with cellular macromolecules.

During incubation of human skin samples *in vitro* with $K_2Cr_2O_7$ (potassium dichromate), the hexavalent salt was reduced to Cr(III) (Table III-9) (Samitz and Katz 1963), possibly by methionine and cysteine (Samitz and Katz 1964). In Table III-9, it can be seen that the majority of the 1.28 mg of Cr(VI) used in each incubation was reduced to Cr(III). The percentage of Cr(VI) reduced to Cr(III) was calculated from the data in Table III-9 as follows:

$$\begin{array}{c}\%\text{ Cr}\\ \text{Reduced}\end{array} = \frac{(\text{Cr(VI) Red. to Cr(III) in mg/g skin})\ (\text{Wt of Wet Skin in g})}{\begin{array}{c}\text{Cr(VI) Red. to Cr(III)}\\ \text{in mg/g skin}\end{array} \times \begin{array}{c}\text{Wt of Wet Skin}\\ \text{in g}\end{array} + \begin{array}{c}\text{Cr(VI) found}\\ \text{in mg}\end{array}} \times 100$$

In the six experiments described in Table III-9, an average of 68% of the initial 1.28 mg of hexavalent chromium was found to be reduced.

Table III-9 Reduction of Hexavalent Chromium by Skin[a]

Wet skin (g)	Cr(VI) found (mg)	Total chromium found (mg)	Cr(VI) reduced to Cr(III) (mg/g skin)
0.844		1.28	
0.786		1.28	
0.836		1.28	
0.849		1.29	
0.786		1.29	
0.888		1.28	
0.800	0.43		1.06
0.791	0.47		1.03
0.852	0.44		0.98
0.847	0.37		1.08
0.722	0.46		1.14
0.960	0.27		1.05

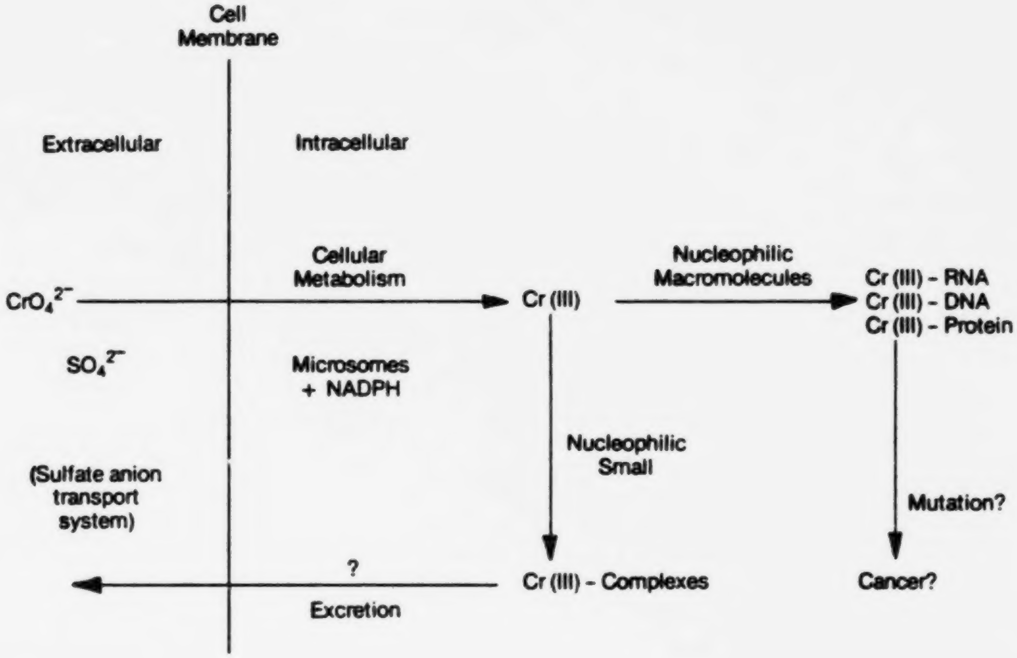
[a] All samples initially contained 1.28 mg Cr(VI).

Adapted from Samitz and Katz (1963).

Further confirmation of the reduction of Cr(VI) to Cr(III) was obtained when breast, liver and thyroid tissues were treated with dichromate or Zenker's solution (Cr(VI)) (Gutierrez et al. 1976). The tissue exhibited electron paramagnetic response (EPR) spectra characteristic of Cr(III).

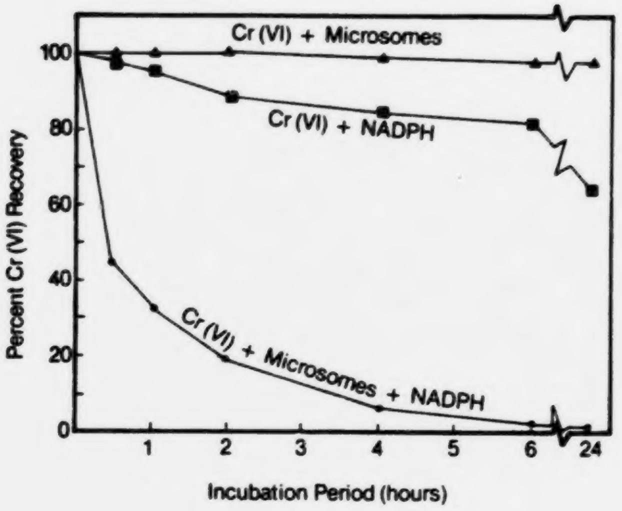
A general scheme for the cellular metabolism of chromium and its interaction with cellular constituents is given in Figure III-1. Chromate (Cr(VI)), which is a tetrahedral anion at physiological pH, traverses the biological membrane by diffusion or facilitated transport, possibly via an anion transport system (Alexander et al. 1982). It is then reduced to Cr(III) intracellularly by the microsomal electron transport cytochrome P-450 system in the presence of NADPH (Gruber and Jennette 1978) (Figure III-2). From Figure III-2, it can be seen that the incubation of chromate (Cr(VI)) with microsomes from rat liver (1.5 mg/mL) in the absence of NADPH resulted in nearly complete recovery of Cr(VI) after a six-hr incubation. This result indicated that chromate did not oxidize any components of the microsomal fraction under these conditions. NADPH reduced chromate (Cr(VI)) in the absence of microsomes at a very slow rate (less than 20% reduction of chromate after six hours of incubation). Incubation of chromate (Cr(VI)) in the presence of microsomes and NADPH resulted in more than 98% reduction of chromate, suggesting that an NADPH-requiring enzyme or enzyme complex was involved in the intracellular reduction of chromate.

After intracellular reduction of Cr(VI) to Cr(III), Cr(III) reacts with nucleophilic ligands of the both high and low molecular weight. The


Cell
Membrane
Extracellular
Intracellular
Cellular
Metabolism
CrO_4^{2-}
Cr (III)
Nucleophilic
Macromolecules
Cr (III) - RNA
Cr (III) - DNA
Cr (III) - Protein
SO_4^{2-}
Microsomes
+ NADPH
Nucleophilic
Small
(Sulfate anion
transport
system)
Mutation?
?
Cr (III) - Complexes
Cancer?
Excretion

Adapted from Jennette (1979). The complexes of chromium with nucleophiles are assumed to be octahedral complexes with chromium as Cr(III). Complexes with RNA have been reported by other investigators (Fuwa et al. 1960, Huff et al. 1964) and were added to the Cr(III) complexes with nucleophilic macromolecule.

Figure III-1 Metabolic Fate of Chromium at the Cellular Level



Adapted from Gruber and Jennette (1978). All solutions contained 0.05 M TRIS.HCl, pH 7.4, CrO_4^{2-} = 4.0×10^{-4}. Test solutions also contained:

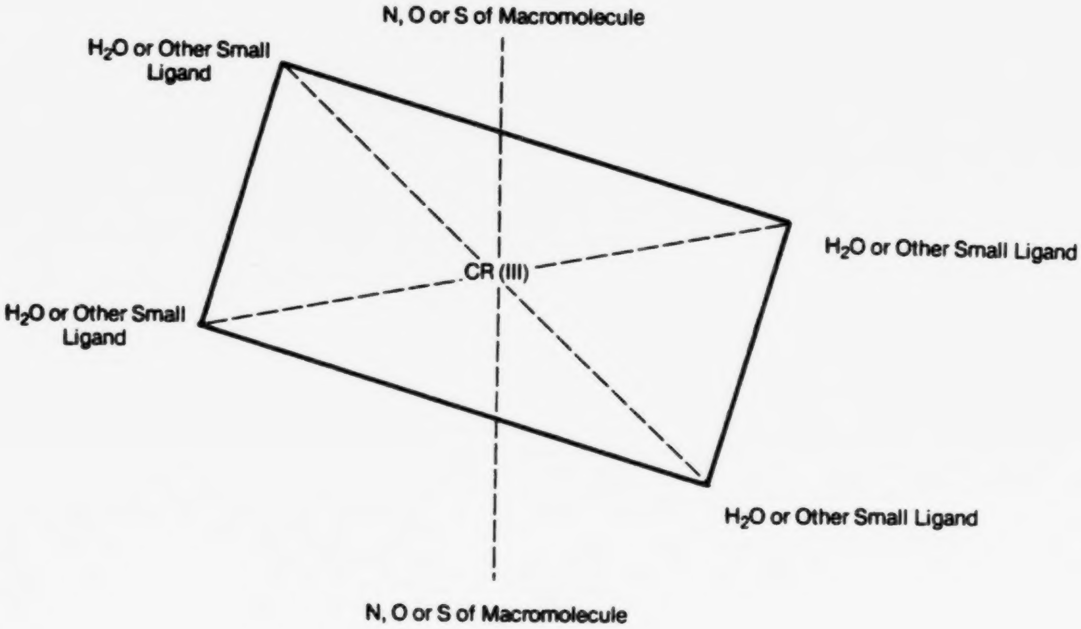
- ▲ : 1.5 mg/mL microsomal protein
- ■ : 1.44×10^{-3} M NADPH
- ● : 1.5 mg/mL microsomal protein + 1.44×10^{-3} M NADPH

Microsomes were isolated from rats previously injected with sesame oil. All incubations were done at 37°C with shaking.

Figure III-2 Time Course Studies on the Recovery of Chromate from Solutions Incubated with Rat Liver Microsomes and/or NADPH

coordination binding probably involves nitrogen, oxygen or sulfur ligands. The octahedral complex formed between Cr(III) and macromolecules may take the form of cross-linked bonds between DNA strands (Gruber and Jennette 1978, Jennette 1979), within the RNA molecule (Fuwa et al. 1960, Huff et al. 1964), or between or within protein molecules (Bowes and Kenten 1948, 1949). A possible octahedral configuration for cross-linking of Cr(III) with macromolecules is shown in Figure III-3. In Figure III-3, of the six binding sites available in octahedral Cr(III), the four binding sites in the plane of the octahedron are shown complexed to H_2O or other small ligands, whereas the two binding sites above and below the plane are thought to complex to the nitrogen, oxygen or sulfur atoms of cellular macromolecules. Our knowledge of specific interactions of chromium with proteins and nucleic acids is rather limited. However, recent work by Tsapakos et al. (1981) indicates that the DNA cross-links formed in nuclei from the livers and kidneys of rats treated with sodium dichromate may be mostly DNA-protein in nature. (See Section VII, Mechanisms of Toxicity, and Table VII-4.)

Glucose tolerance factor is more widely distributed than other forms of chromium and is thought to potentiate insulin action, possibly by forming a complex between sulfhydryl groups on the cell membrane and on the A-chain of insulin (Mertz 1974, 1976). However, the metabolic fate of the chromium in GTF is not known. Another low molecular weight complex of Cr(III) is synthesized in liver (Yamamoto et al. 1981). This complex was also found in plasma, feces and urine, and may play a role in detoxification, transport and excretion of the metal; it is not identical with GTF.


N, O or S of Macromolecule
H_2O or Other Small Ligand
H_2O or Other Small Ligand
CR (III)
H_2O or Other Small Ligand
H_2O or Other Small Ligand
N, O or S of Macromolecule

Adapted from the structure for glucose tolerance factor from Saner (1980). H_2O or other small ligands have been substituted for amino acids in the plane of the octahedron and the bonding above and below the plane of the octahedron is to macromolecules instead of nicotinic acid as cited by Saner (1980). Tsapakos et al. (1981) have suggested that Cr(III), as an electrophile, is capable of binding to a maximum of six nucleophilic sites in macromolecules. Bonding to only two nucleophilic sites is shown in this figure.

Figure III-3 Proposed Octahedral Complex Between Chromium and Cellular Macromolecules

D. Excretion

Because of analytical difficulties, very little is known about the form in which chromium is excreted. Determinations of chromium in the urine have largely measured total chromium, or analyses have been performed in such a manner (e.g., ashing) that a change in the oxidation state of chromium may have taken place.

The urinary system is the major excretory route for chromium, accounting for 80% or more of chromium excretion. A small amount of chromium absorbed after oral administration is also excreted in the feces, although reports in the literature vary considerably on the amount excreted.

Collins et al. (1961) reported that after intravenous administration of Cr(III) as $^{51}CrCl_3$ to dogs, 25% of the Cr(III) was excreted in the urine within four hours. Less than 0.5% of the dose was excreted in the bile. The doses ranged from 0.005 to 1.9 mg/kg. Urinary excretion accounted for 98% of the amount of Cr(III) excreted within four hours. Collins et al. (1961) also reported that over a four-day period in dogs with bile duct fistulas, 50%, 0.5% and 3.7% of the dose of Cr(III) was excreted in the urine, bile and feces, respectively. Urinary excretion of Cr, therefore, represented 92% of the total amount excreted. Kraintz and Talmage (1952) reported that after administration of 0.2 mg Cr(III) as $^{51}CrCl_3$ via the caudal vein in rats, 40% of the total dose was excreted in the urine within 24 hours. (Based on an average weight of 190 g for the male rats, the dose was 1.05 mg Cr(III)/kg.)

No radioactivity was detected in the feces, which suggested that 100% of excretion was via the urine.

Collins et al. (1961) also administered Cr(VI) as $Na_2{}^{51}CrO_4$ intravenously, and found that 9% of the total dose of Cr(VI) was excreted in the urine within four hours and less than 0.5% of the total dose appeared in the bile. Therefore, of the total amount excreted, urinary excretion accounted for 95%. In dogs with bile fistulas, the excretion values after four days were reported as 20%, 0.9% and 1.2% of the injected dose in urine, bile and feces, respectively. Excretion via the urinary tract represented 95% of the total amount of chromium excreted within four days.

After intravenous administration, chromium is also excreted in the feces, although reports in the literature vary considerably on the percentage. Hopkins (1965) reported that 0.5% to 1.7% of the initial dose of Cr(III) was excreted in the feces of rats eight hours after intravenous administration of 0.1 µg $^{51}CrCl_3$/100 g.

In rats and rabbits, however, Kraintz and Talmage (1952) found no chromium in the feces after one day, whereas Visek et al. (1953) reported up to 20% excretion of chromium in the feces of rats four days after Cr(III) was administered intravenously. It should be noted, however, that contamination of feces with urine is a problem when metabolic cages are used. Nevertheless, the apparent exclusion of contamination of the feces by urine in the Hopkins

(1965) study indicates that some intestinal excretion of absorbed chromium into the feces takes place in the rat.

In dogs, a small fraction of plasma chromium (9% to 12%) is present in a diffusible form (complexed to various small organic molecules). This chromium is filtered at the glomerulus and partially reabsorbed (at least 63% of the amount filtered) in the renal tubule (Collins et al. 1961). The high rate of reabsorption of chromium by the kidney, both under control conditions and during water diuresis, suggests that chromium may be reabsorbed by an active transport mechanism (Davidson et al. 1974). Chromium, when very tightly bound in low molecular weight complexes such as chromium-EDTA, may serve as a glomerular indicator, being freely filtered but not reabsorbed (Stacy and Thorburn 1966).

E. Bioaccumulation and Retention

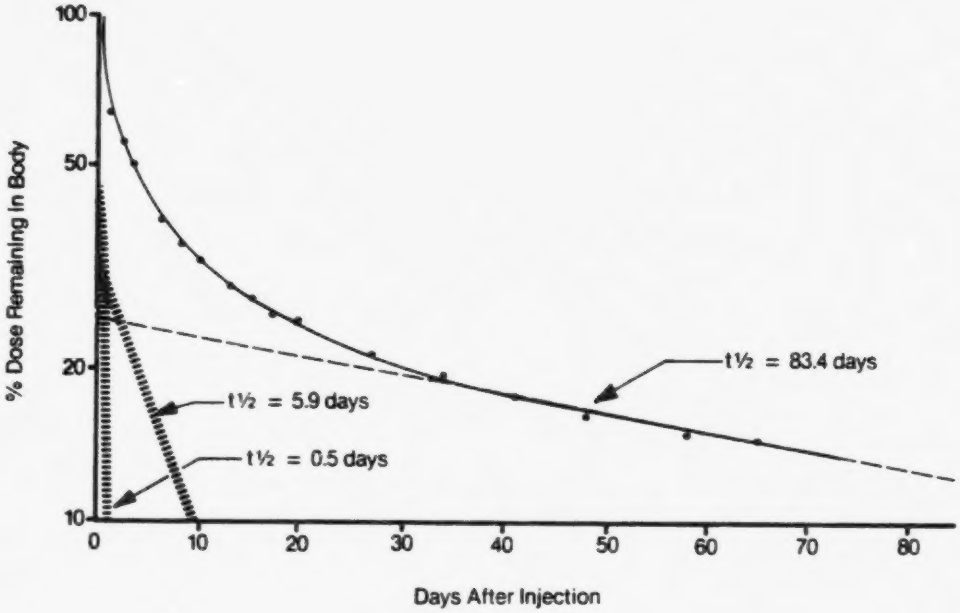
Although little information was available on changes in the specific radioactivity of chromium in different body compartments following administration of ^{51}chromium chloride (Cr(III)), there is sufficient evidence to show that the different compartments exhibit distinctly different turnover kinetics (Lim et al. 1983). In six human subjects a tracer dose of $^{51}CrCl_3$ was injected intravenously, and its distribution was followed for 60 days. Accumulation was found principally in liver, spleen, soft tissue and bone. The data were fitted to a model consisting of a plasma pool in equilibrium with fast, medium and slow compartments; different organs contained varying proportions of each compartment.

Similar results were obtained previously by Mertz et al. (1965). On the basis of polynomial regression analysis, they distinguished at least three kinetic compartments for total body retention, with half-lives of 0.5, 5.9, and 83.4 days, respectively (Mertz et al. 1965) (Figure III-4). Intravenous administration of 0.5 μg of unlabeled chromium on day 9 and 100 μg on day 27 into rats exerted little effect on the rate of tracer excretion from the compartment with the longest half-life (i.e., $t_{1/2}$ of 83.4 days). The pool from which chromium (at least in some systems) enters the plasma following administration of glucose is not readily identified by intravenous, intraperitoneal or oral administration of ^{51}Cr administered as $CrCl_3$ (Cr(III)) (personal communication, W. Mertz 1981). The existence of the various body pools has been inferred from tracer equilibration and excretion studies.

F. Summary

The net absorption of Cr(III) and Cr(VI) in the form of inorganic salts from the intestine is low both in human and in experimental animals. However, the absorption of Cr(VI) across the gastrointestinal tract is greater than that of inorganic Cr(III). In turn, the intestinal absorption of Cr(III) in the form of glucose tolerance factor (GTF) is somewhat greater than that of inorganic Cr(III).

Flameless atomic absorption measurements of chromium levels in human hair suggest that the intestinal absorption of total chromium in infants and young



Adapted from Mertz et al. (1965). Dose: 0.1 μg/100 g as $^{51}CrCl_3 \cdot 6H_2O$. Retention values are the average of four rats.

Figure III-4 Whole-Body Retention of Intravenously Administered Chromium as Percent of Initial Count

children generally exceeds that found in adults. Inorganic Cr(III) administered intravenously or by stomach intubation does not readily cross the placental barrier in rats; however, after administration of GTF to pregnant rats by stomach intubation, Cr(III) is readily recovered from fetal tissues. Cr(III) normally circulates in the plasma bound to transferrin. Cr(VI) readily traverses biological membranes, whereas Cr(III) does not. Cells tend to accumulate chromium to higher levels than those found in plasma, since Cr(VI) appears to be reduced intracellularly to Cr(III), which is then bound to macromolecules. The distribution of chromium in tissues differs, depending on the oxidation state and chemical form of chromium administered. Tissue levels of chromium have been observed to decline with advancing age in mice and humans. Polynomial regression analysis reveals at least three kinetic compartments for the total body retention of chromium in rats. The body pool(s) from which chromium enters the plasma is not readily identified by intravenous or oral administration of radioactive $CrCl_3$ (Cr(III)).

The metabolism of chromium in mammalian species is not well understood. It is complicated by the presence of two oxidation states, Cr(VI) and Cr(III). *In vitro* studies indicate that after traversing cell membranes, Cr(VI) is reduced to Cr(III), which then reacts with cellular macromolecules. The major excretory route for chromium is the urinary system, which accounts for 80% or more of chromium excretion, although the form in which it is excreted is unknown. A small amount of chromium retained after oral administration is also excreted in the feces, although reports in the literature vary considerably on the amount.

IV. HUMAN EXPOSURE

Humans may be exposed to chromium in drinking water, food and air. Detailed information concerning the occurrence of and exposure to chromium in the environment is presented in another document entitled, "Occurrence of Chromium in Drinking Water, Food, and Air" (Letkiewicz et al. 1984). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food and air.

A. Exposure Estimation

This analysis is limited to drinking water, food and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to chromium from sources other than the three considered here. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works and travels, what one eats and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods have not yet been developed to estimate exposure of identifiable population subgroups from all sources simultaneously. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various chromium concentrations. The 70-kg man is used for estimating intake.

1. Drinking Water

Very limited data are available for characterizing human intake of chromium from drinking water provided by public water supplies. Based on the data presented in Letkiewicz et al. (1984), it appears that the drinking water delivered to most (80% to 90%) of the approximately 214 million people in the United States who use public water supplies does not have chromium present above 5 µg/L. Based on the Federal Reporting Data System (FRDS) data, it can be estimated that approximately 215,000 people receive drinking water with chromium levels above 50 µg/L, the current maximum contaminant level (MCL). While all but one of the 18 individual supplies with levels above 50 µg/L have a groundwater source, over half the exposed population (110,000 people) are exposed from the single surface water source.

The data available are too limited to develop a reliable profile of human exposure to various levels of chromium in drinking water below the current MCL.

2. Diet

Chromium intake from the diet for humans is reported to range from 10 to 400 μg/day, with an average of 80 μg/day (Kirk-Othmer 1978). The National Research Council (NRC 1974), using data from Schroeder (1970), indicated that the chromium intake from food is 100 μg/day, ranging from 5 to 500 μg/day. Table IV-1 presents several estimates of daily dietary intake of chromium presented by NRC (1974). It is not clear whether these estimates of dietary intake include drinking water or other water-based beverages. Although absorption of various trivalent chromium salts and compounds (0.1% to 3%) and hexavalent chromium salts (about 10%) is low, ICRP (1974) indicates that at least half of the chromium in food is absorbed.

3. Air

The International Commission on Radiological Protection (ICRP 1974) has estimated that the intake of chromium from air is 0.1 μg/day, with a range of 0.03 to 0.3 μg/day for urban populations. For an adult male inhaling 23 m^3 of air/day, 0.03 to 0.3 μg/day corresponds to a range of air levels of approximately 0.0013 to 0.013 μg/m^3, which is similar to the mean values observed by the National Air Surveillance Network (EPA 1984). No information was available on the absorption rate of inhaled chromium.

Table IV-1 Daily Intake of Chromium in Various Diets

Diet or food (quantity)	Chromium (μg)	Area
Institutional	78	Vermont
Institutional	52	Syracuse, NY
Hospital	101	Vermont
Self-selected	64 (5-115)	Syracuse, NY
Self-selected	200-400	Cincinnati, OH
Vegetarian	11-55	Indiana

Adapted from NRC (1974).

B. Summary

There is a very limited data base on human exposure to chromium from drinking water, food and air. While some information is available to suggest the general range of chromium intake from these three media, it is not possible to determine what proportion of the population has drinking water, dietary and respiratory intakes at various levels. For drinking water, it is possible to estimate the size of the population exposed to levels in public water supplies above 50 µg/L, the current MCL, but not for various levels at or below 50 µg/L. Consequently, a detailed relative source contribution assessment that describes the importance of each medium as a chromium source for different segments of the U.S. population could not be developed.

While it is not possible to develop a detailed profile of human exposure to chromium and the relative source contributions from the three media of interest, it is possible to illustrate the relative importance of each source for various assumed exposure conditions. Table IV-2 presents an illustrative relative source contribution assessment for chromium for the adult male and the newborn formula-fed infant. The absorbed doses from air and food shown in Table IV-2 are based on the typical intakes presented above. Three dose levels are calculated for drinking water based on assumed concentrations of 0.5 and 50 µg/L.

Table IV-2 Illustrative Relative Source Contribution Assessment for Chromium for the Adult Male and the Newborn Formula-Fed Infant

	Adult male[a] (food dose: 0.71 µg/kg/day[b]; air dose: 0.001 µg/kg/day[d])					Newborn formula-fed infant[c] (air dose: 0.003 µg/kg/day[e])			
Drinking water concentration (µg/L)	Drinking water dose (µg/kg/day)[f]	Total dose (µg/kg/day)	% from drinking water	% from food	% from air	Drinking water dose (µg/kg/day)[g]	Total dose (µg/kg/day)	% from drinking water	% from air
0	0	0.71	0	99.9	0.1	0	0.003	0	100
5	0.071	0.78	9.1	90.8	0.1	0.61	0.61	99.5	0.5
50	0.71	1.42	50	50	<0.1	6.1	6.1	>99.9	<0.1

---

[a] Assumes a 70-kg body weight.
[b] Based on reported dietary intake of 100 µg/day and 50% absorption.
[c] Assumes a 3.5-kg body weight.
[d] Based on reported air intake of 0.1 µg/day (which corresponds to 0.004 µg/m^3) and 100% absorption.
[e] Based on an assumed air level of 0.004 µg/m^3, respiratory rate of 0.8 m^3/day and 100% absorption.
[f] Assumes consumption of 2 L/day and 50% absorption.
[g] Assumes consumption of 0.85 L/day and 50% absorption.

The calculations shown in Table IV-2 suggest that for the adult male, food is generally the predominant source, accounting for more than 90% of the absorbed dose for individuals exposed to drinking water with chromium levels below 5 µg/L, which appears to be the majority of the population. Drinking water becomes a source roughly equivalent to food for the adult male only at levels of 50 µg/L or greater, which is the current MCL.

V. HEALTH EFFECTS IN ANIMALS

A. Beneficial Effects

Cr(III) produces beneficial effects when given to animals with chromium deficiency. In rats, inorganic salts of Cr(III) enhance glucose uptake (Mertz et al. 1965), decrease circulating blood cholesterol without affecting the levels of aortic lipids, decrease aortic plaques (Schroeder and Balassa 1965) and increase the life span (Schroeder et al. 1965).

Schroeder and Balassa (1965) have studied the effects of 5 mg/L chromium acetate (Cr(III)) in the drinking water of approximately 50 male and female rats (Long Evans strain) from the time of weaning until death (one to two years) in an environment and on a diet low in chromium (levels not specified). The daily dose may be calculated as follows:

$$\text{Dose in mg/kg/day} = \frac{\text{Cr(III) Concentration in Water, mg/L} \times \text{Water Consumption, L/day}}{\text{Body Weight, kg}}$$

Assuming an average rat weighs 330 g (0.3 kg) and drinks 28 mL (0.03 L) of water per day (Arrington 1972), the daily dose of Cr(III) was 0.42 mg/kg. Compared to controls, serum cholesterol levels were significantly lower in chromium-treated male rats from two age groups (17 months and 24 to 26 months) ($P<0.001$ and $P<0.01$, respectively), whereas in 17-month-old chromium-treated females, serum cholesterol levels were significantly higher than those of the controls ($P<0.05$). However, no significant changes were found in the cholesterol levels of 24- to 26-month-old females. There was only one animal with aortic plaques out of 48 chromium-treated animals, compared to 31 animals with plaques out of 165 control animals ($P<0.01$). Normal levels (not specified) of aortic lipids were found in the chromium-fed group. The results from this study suggest that chromium may function in the catabolism or excretion of cholesterol (Schroeder and Balassa 1965). In another experiment using the same protocol (i.e., 5 mg/L of Cr(III) (0.42 mg Cr(III)/kg/day) in drinking water from weaning to death), Schroeder et al. (1965) studied the life span of chromium-fed rats. From survival curves, the authors found only one significant age difference; at three years of age there were more animals of both sexes alive in the chromium-fed group than in the control group ($P<0.025$). In addition, longevity (defined as the mean age of the last surviving 10% of the population) was greater in the chromium-treated group than in the controls ($P<0.01$ - significant for males only). Chromium-fed females however, had a 7.8% mortality rate resulting from a pneumonia epidemic, compared to 25.6% in the control group (Schroeder et al. 1965).

In male Sprague-Dawley rats maintained on a low-chromium diet, intravenous administration of chromium hexa-urea chloride (2.5 or 5 μg Cr(III)/kg) almost doubled ($P<0.01$) the rate of glucose removal from the blood within two hours bringing the removal rate close to the normal value of 3.5% per min (Mertz et al. 1965).

B. Toxic Effects

1. Acute Effects

Compounds of Cr(III) have low toxicity in man and animals. In rats, LD_{50} values for various Cr(III) salts range from 600 to 2,600 mg Cr(III)/kg (Smyth et al. 1969). The oral LD_{50} for Cr(VI) as $Na_2Cr_2O_7$ in rats is 19.8 mg Cr(VI)/kg (NIOSH 1983).

Last et al. (1979) examined the effect of Cr(VI) aerosols on the trachea of male Sprague-Dawley rats in an attempt to mimic the conditions of acute industrial exposure. Continuous inhalation exposure to sodium chromate at 0.173 mmol Cr(VI)/m^3 (59 mg/m^3) of air caused extensive damage to the tracheal epithelium and submucosal glands within 24 hours. At 0.051 mmol Cr(VI)/m^3 (17.3 mg/m^3) of air, however, there was no effect.

In female mongrel dogs weighing 8 to 16 kg, injection of 1.25 mg (total dose) (0.08 to 0.16 mg/kg) of potassium dichromate (Cr(VI)) into the renal artery caused preferential damage to the first third of the proximal convoluted tubule within 72 hours (Nicholson and Shepherd 1959). This effect, known as dichromate nephrosis, was characterized by increased urinary excretion of glucose and phosphates.

Intraperitoneal administration of massive doses (10 to 20 mg/kg) of sodium chromate (Cr(VI)) to male Wistar rats has also produced selective changes in the proximal convoluted tubules of the kidney (Evan and

Dail 1974). The sequence of changes leading to nephron damage was: (1) swelling and loss of microvilli (2) formation of intracellular vacuoles of varying sizes (3) mitochondrial swelling and (4) cytoplasmic liquefaction followed by desquamation. The first change was noted six hours after 20 mg/kg dose and 24 hours after a 10 mg/kg dose. The degree of cellular degeneration was the same for both dosage levels by 48 hours; however, more cells showed advanced degeneration at the higher than at the lower dose.

No other studies on the acute effects of chromium were found in the available literature.

2. Subchronic and Chronic Effects

Male and female albino rats (Sprague-Dawley, 34-days-old) can apparently tolerate up to 25 mg/L of either trivalent chromium, as chromium chloride ($CrCl_3 \cdot 6H_2O$) or hexavalent chromium as potassium chromate (K_2CrO_4) in their drinking water for one year (MacKenzie et al. 1958). Assuming a daily water intake of 28 mL and an average weights of 375 g for males and 290 for females (Arrington 1972), the daily doses of Cr were 1.87 and 2.41 mg/kg/day for male and female rats, respectively. At concentrations of 0 to 5 mg/L of Cr(VI) (0 to 0.37 mg/kg/day for male rats and 0 to 0.48 mg/kg/day for female rats), relatively little chromium was found in any tissue. However, when the animals received between 5 and 10 mg/L of Cr(VI) (0.37 to 0.75 mg/kg/day for males and 0.48 to 0.97 mg/kg/day for females), an appreciable increase in the concentration of chromium was found in liver, kidney, bone and spleen. No significant differences in either weight, food and water consumption or blood analyses were observed between experimental groups and controls. At 25 mg/L (1.87 and 2.41 mg/kg/day for male and female rats, respectively), neither Cr(III) nor Cr(VI) produced a significant difference in either weight gain or food consumption. In addition, no gross changes in appearance or pathological changes in the blood or other tissues were observed. Water intake, however, was decreased in animals receiving hexavalent chromium: females drank 77% and males 84% of the volumes ingested by the controls. However, relative to control values, the increase in tissue chromium levels of animals receiving Cr(VI) was approximately 12.5 times that of animals receiving Cr(III).

Six-week-old female dogs (five dose groups with two animals per group) given 0.45 to 11.2 mg/L of Cr(VI) as K_2CrO_4 in their drinking water for four years also showed no abnormalities in their physical condition, food consumption or growth rate (Anwar et al. 1961); the weights of the liver, kidney and spleen also remained normal. Assuming that an average dog weighs 13 kg and drinks 350 mL of water/day (Arrington 1972), the daily dose was between 0.012 and 0.30 mg/kg of Cr(VI), or a total dose after four years of between 17.5 mg/kg and 438 mg/kg of Cr(VI). No significant changes (statistical analyses not provided). were found in either routine urinalysis for albumin, acetone, bile pigments, glucose and erythrocytes, or in blood analysis for erythrocyte count, total or differential leukocyte counts and hemoglobin. Chromium accumulation was observed in the liver, kidney and spleen but appeared to be independent of the concentration of chromium received by the dogs in their drinking water.

Gross and Heller (1946) used growth curves to determine the maximum nontoxic levels of either potassium chromate (K_2CrO_4) or zinc chromate ($ZnCrO_4$)--hexavalent salts--permissible in the diets of mice and rats. Administration of up to 500 mg/L of K_2CrO_4 (13.4 mg (Cr(VI))/L)in drinking water to mature white mice did not affect their general condition nor their reproductive capabilities. Assuming mature mice weigh 30 g and drink 5 mL of water/day (Arrington 1972), the daily dose was 83 mg/kg (22 mg Cr(VI)/kg). However, the exact age, sex, and weight of the animals, the period of exposure and the total dose of K_2CrO_4 received were not reported. In young male and female albino rats, 300 mg/L of K_2CrO_4 (80 mg Cr(VI)/L) in drinking water (8.3 mg/kg/day, based on an average final weight of male and female rats of 270 g and assuming the rats drank 28 mL/day (Arrington 1972)) produced no toxicity over a two-month period. However, at 134 mg Cr(VI)/L 28 mL (14.4 mg/kg/day, based on an average final weight of 260 g, and a water consumption of 28 mL), a slight roughness in the coats of the animals was apparent (Gross and Heller 1946).

In young male (initial weight approximately 80 g to 270 g) and female (initial weight approximately 75 g to 240 g) albino rats, administration of K_2CrO_4 as 0.125% of the diet produced no toxicity over a 2- to 2.5-month period (Gross and Heller 1946). In this instance, assuming an average rat weighs 250 g and eats 12 g of food per day (Arrington 1972), the daily dose of K_2CrO_4 was 60 mg/kg (16 mg Cr(VI)/kg/day). However, doses of 0.25% K_2CrO_4 (120 mg K_2CrO_4/kg/day or 32 mg Cr(VI)/kg/day) to 1.0% (480 mg/kg/day) of K_2CrO_4 (128.6 mg Cr(VI)/kg/day) over a 24- to 90-day period produced a deterioration in the animals' condition which included: diarrhea, rough, dirty coats and tails and subnormal reproductive capability including sterility.

In contrast, $ZnCrO_4$ was more toxic when administered to the young albino rats in food. Even at the lowest dose (0.125% of their food, equivalent to 60 mg/kg/day of $ZnCrO_4$ or 17.22 mg Cr(VI)/kg/day), the general condition of the rats was subnormal; coats and tails were rough and dirty and the animals were sterile. At the highest level (i.e., 480 mg $ZnCrO_4$/kg/day or 137.8 mg Cr(VI)/kg/day), rats became emaciated, with stunted growth and rough, dirty coats and tails (Gross and Heller 1946). However, in half-grown albino rats and mature white mice, 1% $ZnCrO_4$ (480 mg/kg/day) in the food did not affect either the general condition of the animals or their reproductive capabilities, although there was a slight roughness in the coats of the rats.

Thus for rats of all ages, 0.125% (60 mg/kg) K_2CrO_4 (16 mg Cr(VI)/kg/day) was the maximum nontoxic level permissible in the food. The equivalent level of $ZnCrO_4$ for mature white rats and mice was 1% (480 mg $ZnCrO_4$/kg/day or 137.8 mg Cr(VI)/kg/day), but for young rats it was less than 0.125%.

Chromium oxide (Cr(III)) was fed to two groups of rats (male and female) for 90 days at average cumulative doses of 74 and 170 g/kg bw

(Ivankovic and Preussmann 1975). A dose-dependent reduction in the weights of the liver and spleen was established, although no significant differences (no P value given) in body weights were seen between the experimental group and controls. In addition, blood samples from control and chromium-treated animals exhibited no differences in hemoglobin level, erythrocyte count, total and differential leucocyte count or serum protein and blood sugar levels.

Hunt and Nielsen (1979) reported that chromium acetate (500 mg Cr(III)/kg food) acted synergistically with vanadium, causing small amounts of vanadium (5 mg/kg food) to become toxic to day-old chicks within four and one-half weeks. When examined, the cockerels were stunted and somewhat emaciated and had enlarged gall bladders and livers as well as disorganized epiphyseal plates. High dietary levels of chromium (500 to 2,000 μg/g), however, were effective in overcoming growth depression and mortality in rats fed 20 μg vanadium/g of diet (Wright 1968, as reported in NAS 1980b).

Johansson et al. (1980) have investigated the effects of inhalation of chromium dust on the tracheas of male rabbits weighing 2.2 to 2.7 kg. The animals were exposed to chromium dust particles at levels of 1.9 mg/m^3 and 0.5 mg/m^3 for six hours per day, five days per week, for four weeks. Following exposure, alveolar macrophages were collected and analyzed for phagocytic activity. Inhalation of 1.9 mg $chromium/m^3$ increased phagocytic activity significantly ($P<0.02$), whereas inhalation of 0.5 mg $chromium/m^3$ did not ($P>0.05$).

Mathur et al. (1977) have compared the toxicity of Cr(III) and Cr(VI) compounds in ITRC colony-bred male rabbits (average body weight 1.5 kg). Daily doses of either chromium nitrate (Cr(III)) or potassium dichromate (Cr(VI)) (2 mg/kg body weight/day) were administered intraperitoneally for up to six weeks. Groups of animals were sacrificed at three and six weeks, 72 hours after their last injection of chromium. The 72 hour period was necessary to allow excretion of most of the unbound chromium from the body. All animals appeared normal up to the time of sacrifice.

At three weeks, the brains of the Cr(III)-treated animals (average total dose: 63 mg) displayed neuronal degeneration in the cerebral cortex, chromatinolysis, and nuclear changes in the neurons. The kidneys exhibited congestion and extravasation of red blood cells in the intertubular spaces as well as tubular necrosis accompanied by infiltration with mononuclear cells. No pathological changes were noted in the myocardium after three weeks (Mathur et al. 1977).

After six weeks of Cr(III) (average total dose: 126 mg), additional changes such as marked neuronal degeneration in the cerebral cortex, neuronophagia, neuroglial proliferation and meningeal congestion were noted. No further changes were noted in the kidney after six weeks. Marked congestion and degenerative changes of the myocardial muscle fibers were seen, particularly in the subendocardial region. In addition, the cytoplasm of the degenerated muscle fibers appeared vacuolated and contained pyknotic nuclei (Mathur et al. 1977).

Following three weeks of Cr(VI) treatment (average total dose: 63 mg), the brain appeared congested as the result of perivascular infiltration by inflammatory cells. Cerebral cortex neurons exhibited pyknotic nuclei and dissolution of Nissl substance; neuronophagia and focal neuroglial proliferation were also observed throughout the cerebral cortex. Marked congestion was also seen in the kidneys and the walls of small blood vessels; the latter were thickened and hyalinated, particularly near the glomeruli. Necrosis and irregular desquamation of the epithelium of the convoluted tubules were observed, as was the infiltration of red blood cells into the intertubular spaces. There were no pathological changes in the myocardium after three weeks (Mathur et al. 1977).

After six weeks of Cr(VI) administration (average total dose: 126 mg), no further changes were seen in the brain and kidney but marked congestion and degenerative changes of the myocardial muscle fibers were observed, particularly in the subendocardial region. In addition, the cytoplasm of the degenerated muscle fibers appeared vacuolated and contained pyknotic nuclei (Mathur et al. 1977).

Behari et al. (1978), in experiments similar to those of Mathur et al. (1977), also evaluated the effects of Cr(III) and Cr(VI) in the same strain of rabbits (age unspecified; weight 1.5 ± 0.2 kg). Daily intraperitoneal administration of 2 mg/kg body weight of chromium nitrate (Cr(III)) for three weeks produced constriction (referred to as congestion by the authors) of the blood vessels and degenerative changes in the seminiferous epithelium. After six weeks, there was a complete absence of spermatocytes in the lumen.

Rabbits receiving K_2CrO_4 (Cr(VI)), 0.2 mg/kg/day, administered intraperitoneally, also developed edema of the interstitial tissues of the testis after three weeks, followed by constriction (referred to as congestion by the authors) of the blood vessels at six weeks. In addition, the seminiferous tubules were devoid of spermatocytes at six weeks (Behari et al. 1978).

No other studies on the subchronic and chronic effects of chromium were found in the available literature.

3. Teratogenicity

The literature available on possible fetal damage caused by chromium compounds is limited. There were no studies on teratogenic effects produced by chromium following ingestion.

The placenta appears to be highly selective in its permeability to the various forms of chromium. Inorganic Cr(III) administered as $^{51}CrCl_3$ (chromium chloride) intravenously or by stomach intubation does not appreciably cross the placental barrier in rats (Mertz et al. 1969). However, Cr(III) administered by stomach intubation to pregnant rats in the form of GTF (obtained from yeast) is readily recovered from the fetus (Mertz and Roginski 1971).

When sodium dichromate (Cr(VI)) was injected onto the chorioallantoic membrane of eight-day-old chick eggs, embryonic abnormalities such as achondroplasia, parrot [illegible], and slight stunting in growth were observed in 60% of the chick embryos (Ridgway and Karnofsky 1952). The LD_{50} of Cr(VI) injected into eggs was estimated at 0.06 mg/egg for four-day-old embryos and 0.17 μg/egg for eight-day-old embryos. In contrast, application of chromium nitrate (Cr(III), dose unspecified) to the chorioallantoic membrane did not produce teratological effects. Consistent with these observations were the higher LD_{50} values for Cr(III). Following injection of Cr(III) into the yolk sac, the LD_{50} was estimated at 0.80 mg/egg for four-day old embryos and 1.25 μg/egg for eight-day old embryos (Ridgway and Karnofsky 1952).

Gilani and Marano (1979) have examined the teratological potential of hexavalent chromium trioxide (CrO_3, "chromic acid"). The oxide was dissolved in physiological saline and injected into the air sacs of chicken eggs after zero, one, two, three and four days of incubation. Embryos were examined on the eighth day of incubation and a prominent dose-response relationship was observed for embryolethal effects over the range of 0.002 to 0.05 mg/egg. Malformations noted consisted of reduced body size, microphthalmia, short and twisted limbs, everted viscera, ectopic heart, short and twisted neck, edema and exencephaly. Limb defects were the most common anomalies observed. Since these experiments entailed placement of chromium within the embryo's environment, however, their significance in relation to the natural exposure of mammals to chromium compounds is questionable.

4. Mutagenicity

Chromium compounds possess the ability to cause morphological changes in mammalian cells. Tsuda and Kato (1976) detected chromosomal aberrations in hamster embryo cells that had been exposed *in vitro* to potassium dichromate ($K_2Cr_2O_7$) (Cr(VI)) (0.1 to 0.5 µg/mL culture medium). The chromosomal lesions produced included abnormal metaphases, gaps, chromatid breaks and exchanges, as well as deletions and fragmentations. Raffetto et al. (1977) examined the changes produced in tertiary mouse fetal cell cultures following exposure to equitoxic doses of chromium chloride (Cr(III)) (0.4 µg/mL) and potassium dichromate (Cr(VI)) (0.1 µg/mL). Both compounds produced similar morphological changes; however, more chromosomal aberrations were noted in cells treated with Cr(VI) than in those treated with Cr(III) (Table V-1). Cells treated with Cr(VI) also exhibited unscheduled DNA synthesis whereas those treated with Cr(III) did not.

The effects of two Cr(VI) compounds, potassium chromate (K_2CrO_4) and potassium dichromate ($K_2Cr_2O_7$), on bone marrow cells have been studied in mice and white, non-inbred rats, respectively (Wild 1978; Bigaliev and Elemesova 1978). Two intraperitoneal injections, 24 hours apart, of 24.25 and 48.5 mg of K_2CrO_4 (Cr(VI))/kg body weight in mice (both sexes; weight 26 to 35 g) produced a significant increase (alpha <0.01) in the number of micronucleated

Table V-1 Chromosome Analysis of Mouse Fetal Cells Treated with Cr(III) or (Cr(VI))

Treatment	No. of cells analyzed	No. of cells with chromosmal aberrations				
		Total No.	No. of breaks	No. of fragments	No. of acentrics	No. of dicentrics
First Experiment: 48-hr Exposure						
Controls	50	0	0	0	0	0
Cr(III) 0.1 μg/mL	40	0	0	0	0	0
Cr(VI) 0.1 μg/mL	48	12[a]	8	4	0	0
Second Experiment: 96-hr Exposure						
Controls	50	0	0	0	0	0
Cr(III) 0.04 μg/mL	41	3	1	2	0	0
0.4 μg/mL	51	15[a,c]	4	7	3	1
Cr(VI) 0.015 μg/mL	48	12[a,c]	0	12	0	0
0.1 μg/mL	46	25[a,b,c]	3	21	1	0

[a] Denotes a significant difference ($P<0.05$) from corresponding control values.
[b] Denotes a significant difference ($P<0.05$) from values of the equitoxic concentration of Cr(III).
[c] Denotes a significant difference ($P<0.05$) from values of the lower concentration.

Adapted from Raffetto et al. (1977).

polychromatic erythrocytes, whereas two intraperitoneal injections, 24 hours apart, of 12.12 mg K_2CrO_4 (Cr(VI))/kg had no significant effect (Wild 1978). A single dose of potassium dichromate (Cr(VI)) administered intraperitoneally to rats at 15 mg/kg (weight of rats not stated) produced a significant increase in the number of bone marrow cells with metaphasic chromosomal aberrations (Bigaliev and Elemesova 1978).

In a study by Nishimura and Umeda (1978), FM3A cells derived from C3H mouse mammary carcinomas were exposed in vitro to potassium dichromate, chromic acid, potassium chromate or chromium sulfate (doses not stated). Of the Cr(VI) compounds, potassium dichromate and chromic acid induced both chromosomal aberrations and 8-azaguanine-resistant mutations, whereas potassium chromate enhanced only chromosomal changes; chromium sulfate (Cr(III)) had no effect.

Majone and Levis (1979) have evaluated the mutagenicity of hexavalent potassium dichromate and sodium dichromate in Chinese hamster cells in vitro. Both compounds induced mitotic delays that were proportional to the dose of chromium. Chromosomal aberrations were also produced; these were primarily chromatid gaps, breaks, and interchanges and were dose-dependent over a range of 0.1 to 1.0 μg Cr(VI)/mL. Sister chromatid exchanges were used as an index of chromosomal damage. Both Cr(VI) compounds increased the frequency of chromosomal aberrations but were weak inducers of sister chromatid exchange.

DiPaolo and Casto (1979) have also demonstrated morphological transformations (e.g., criss-crossing and piling up of cells) using cultured Syrian hamster embryo cells exposed to sodium chromate (Cr(VI)) at 0.1, 0.5 and 1.0 µg/mL. The frequencies of transformation were 0.7%, 2.10% and 3.48%, respectively.

Newbold et al. (1979) have exposed Chinese hamster cells to potassium dichromate (Cr(VI)) (0.35 to 0.78 µg/mL) and zinc chromate (Cr(VI)) (1 to 4 µg/mL) using 8-azaguanine-resistance to measure mutagenicity. Both compounds were mutagenic in a dose-dependent manner at all levels tested. Chromic acetate (Cr(III)) (50 to 200 µg/mL) and the relatively insoluble lead chromate (Cr(VI)) (5 to 10 µg/mL) were not mutagenic, however, even at doses that were approximately 200 and 10 times higher, respectively, than the maximum dose at which cell survival was measurable with potassium dichromate. The mutation frequency in the presence of potassium dichromate increased in a linear fashion with increasing lethality when only subthreshold (minimally lethal) doses were considered. At higher doses it became harder to recover mutants due to the increased incidence of cell death.

Both potassium chromate (Cr(VI)) (0.15 µg/mL) and chromium chloride (Cr(III)) (50 µg/mL) induced chromosomal aberrations and mitotic alterations in the pseudodiploid Chinese hamster ovary cell line (Majone and Rensi 1979). Levis and Majone (1979), also using the pseudodiploid line, evaluated 11 water-soluble salts of Cr(III) and Cr(VI). Giant cells were produced by exposure to both Cr(VI) and Cr(III) compounds. The frequency of chromosomal aberrations was increased tenfold after exposure to 1.0 µg/mL Cr(VI) as sodium chromate, but only doubled after treatment with 50 to 150 µg/mL Cr(III) as chromium nitrate. A significant number ($P<0.001$) of sister chromatid exchanges were seen after exposure to 0.1 µg/mL of the Cr(VI) compounds potassium dichromate, sodium dichromate and chromic acid, but not in response to 150 µg/mL of trivalent chromium nitrate or chromium potassium sulfate.

The difficulty of observing mutagenic effects of Cr(III) may be related to its slight uptake by cells under most conditions. Warren et al. (1981) studied the mutagenicity of a series of hexacoordinate Cr(III) compounds and concluded that, in the proper ligand environment, the metal possesses considerable genetic toxicity.

Numerous experimenters have used Escherichia coli in attempts to determine the mutagenicity of chromium compounds. Venitt and Levy (1974) reported that the soluble chromates of sodium, potassium and calcium (Cr(VI)), at doses of 0.05, 0.10, and 0.20 µmol/agar plate, stimulated mutagenesis in E. coli. In the E. coli Hs30R test system (which utilizes arginine revertants), Nakamuro et al. (1978) found that the Cr(VI) salts potassium dichromate and potassium chromate and the trivalent chromium triacetate produced significant ($P<0.01$) numbers of chromosomal aberrations following treatment at concentrations of 0.5×10^{-6} M, 4×10^{-6} M and 1.6×10^{-5} M, respectively.

Other researchers have studied the mutagenic characteristics of a variety of chromium compounds in Salmonella typhimurium. Throughout a series of experiments, Cr(VI) compounds (sodium dichromate, chromic acid, calcium chromate and potassium chromate) consistently produced a marked increase in His^+ revertants at concentrations of 10 to 200 μg/plate, whereas Cr(III) compounds (chromium potassium sulfate and chromium chloride) did not (Petrilli and De Flora 1977, 1978a). It was suggested that Cr(VI) compounds interacted directly with bacterial DNA by causing both frame-shift mutations and base-pair substitutions (Petrilli and De Flora 1977).

In an attempt to determine whether Cr(III) could be metabolically oxidized to the mutagenic form, Cr(VI), the *S. typhimurium* test system was used with or without the following additions: rat liver, lung or muscle microsomal fractions, rat muscle mitochondria, or human serum, plasma, or erythrocyte lysates (Petrilli and De Flora 1978a). None of the preparations revealed metabolic activation of the soluble trivalent compounds chromium potassium sulfate, chromium nitrate, chromium chloride, neochromium and chromium alum, even when supplemented with ATP or oxidized glutathione. Because rat liver microsomal preparations inhibited Cr(VI) mutagenicity (presumably by reduction of Cr(VI) to Cr(III)), Petrilli and De Flora (1978b) also evaluated the effects of a variety of reducing agents (ascorbic acid, sodium sulfite) and metabolites (GSH, DPNH and TPNH) on the mutagenic activity of Cr(VI). All of these compounds prevented Cr(VI) mutagenicity. In addition, Petrilli and De Flora (1978b) found that potassium permanganate (an oxidizing agent) inhibited the ability of rat liver microsomal preparations to prevent Cr(VI) mutagenicity. Lofroth (1978), utilizing a *Salmonella*/microsome test system, also found that both chromate and dichromate (Cr(VI)) compounds were metabolized by a NADPH-dependent microsomal enzyme system which resulted in the decreased mutagenic activity of the compounds.

Welding fume particles have been checked for mutagenicity against both E. coli and S. typhimurium by Hedenstedt et al. (1977). Welding material can be separated into two major groups: (a) mild steel, i.e., low alloyed iron with about 1% silicon, 1% carbon and 1% manganese; and (b) stainless steel, i.e., high alloyed metal varying in composition but typically containing 15% to 25% chromium, 8% to 15% nickel and up to 3% molybdenum.

The particles were mutagenic against both types of bacteria at unspecified concentrations. The effect, however, was decreased or eliminated when a rat liver post-mitochondrial fraction was added to the test system.

Nestmann et al. (1979) evaluated the mutagenic potential of lead chromate (Cr(VI)) using: the Salmonella typhimurium His^+ reversion assay at 0.2 mg/plate, the Saccharomyces cerevisiae assay for mitotic recombination at 0.0625 mg/mL, and the E. coli Trp^+ reversion fluctuation test over a range of 5×10^{-6} to 1×10^{-5} M. All tests were positive at the concentrations indicated. Further studies using lead chloride and chromium trioxide (Cr(VI)) indicated that chromium was responsible for the observed mutagenic effect.

Kanematsu et al. (1980) used a recombination-repair assay with Bacillus subtilis to evaluate the mutagenicity of hexavalent chromic acid (0.01 M), potassium chromate (0.005 M) and potassium dichromate (0.005 M), and trivalent chromium potassium sulfate and chromium sulfate, the latter two at unreported concentrations. All Cr(VI) compounds were mutagenic, whereas the Cr(III) compounds were not.

Mass and Lane (1976) evaluated the effect of hexavalent calcium chromate and sodium chromate _in vitro_ on the ciliated cells of tracheal epithelium obtained from female Wistar-Lewis rats. At concentrations above 5 µg chromate/mL, cytotoxicity (i.e., loss of normal columnar orientations, grossly degenerated exfoliated cells) was directly proportional to the product of the concentration and the duration of exposure. Concentrations above 1 mg/mL were lethal within 6 hours, whereas doses between 10 and 100 µg/mL produced cell death within 24 hour.

From the data presented in this section it is apparent that Cr(VI) compounds are mutagenic in both mammalian and microbial systems, whereas information on the mutagenicity of Cr(III) is conflicting. The mutagenicity of Cr(VI) can be inhibited by reduction, as apparently occurs in rat liver microsomes in the presence of NADPH (Lofroth 1978). The biological effect of Cr(VI) appears to depend on where it is reduced--in extracellular spaces, in the cytoplasm or in the nucleus (Lofroth 1978). The studies of Gruber and Jennette (1978) indicate that after entry of the frequently more soluble Cr(VI) compounds into the cell, the enzymes bound to the endoplasmic reticulum reduce it to Cr(III), which suggests that Cr(III) may be the ultimate intracellular agent.

5. Carcinogenicity

The carcinogenicity of chromium compounds in experimental animals has been widely investigated (Sunderman 1971; Mertz 1969), although only one ingestion study was found in the available literature (Ivankovic and Preussmann 1975). In studies where cancers were found, most were sarcomas that formed at the site of administration (Payne 1960). Attempts to initiate cancerous growths under conditions of exposure simulating those occurring environmentally have not been successful.

Ivankovic and Preussmann (1975) were unable to produce any carcinogenic effects when they added chromium oxide (Cr_2O_3) (Cr(III)) to the food of male and female rats (inbred BD strain, 100 days old, weighing an average of 200 g) for a period of two years (600 feeding days). The diets contained 1%, 2% or 5% Cr_2O_3. Fifteen to twenty rats per dose were used. The average cumulative doses of Cr_2O_3 were 0.6, 1,2 or 3.0 g/kg, corresponding to average daily doses of 293, 586 or 1,466 mg Cr(III)/kg/day for each group. No significant (P value not given) in body weights or median survival times were observed between the experimental groups and controls. No macroscopic or histological findings were attributed to the chromium treatment.

Nettesheim et al. (1970) exposed germfree-derived, specific pathogen-free mice of the C57BL/6 inbred line to Cr_2O_3 dust (25 mg/m^3) for 5.5 hours per day, 5 days each week, for up to 18 months and observed no cancerous growths.

Baetjer et al. (1959b) reported that exposure of three different strains of mice (A, Swiss and C57 Black) and rats (mixed strain from Wistar and

McCollum stock) to 1 to 3 mg/m^3 of mixed chromium dust (Cr(III) and Cr(VI)) in a dust chamber did not induce bronchogenic carcinomas nor did it increase the incidence of benign tumors. Steffee and Baetjer (1965) studied the effects of inhalation and/or intratracheal injection of mixed chromium dust (Cr(III) and Cr(VI)) and two Cr(VI) compounds, zinc chromate and lead chromate, in rabbits (both sexes and various stocks), guinea pigs (both sexes and various stocks), mice (A strain) and rats (Wistar strain). Intratracheal administration of a 1% solution of zinc chromate produced a high incidence of changes in the alveolar epithelium of all species. Alveolar and interstitial inflammation was significantly ($P<0.01$) increased in the guinea pig following the inhalation of 3 to 4 mg of chromium dust (chromic acid)/m^3, as well as in mice treated intratracheally with zinc chromate. Granulomata were seen in a significant number of chromate-exposed rabbits, guinea pigs, and rats, although there were no malignancies. Unfortunately, dose levels were not specified for all compounds.

Intrabronchial implantation of pellets of either calcium chromate (Cr(VI)) or a chromate-chromite mix in rats produced bronchogenic carcinomas (Laskin et al. 1970). Chromic trioxide (Cr(III)) produced hepato-cell carcinomas, although unlike the animals treated with the two Cr(VI) compounds, the Cr(III)-treated animals did not show evidence of atypical squamous metaplasia of the bronchus. No indication of dose levels was given for any of these compounds.

Recently, Knudsen (1980) studied the carcinogenic potential of potassium chromate (K_2CrO_4) (Cr(VI)) using a new mammalian spot test in pregnant mice

(C57BL/6J/BOM). The system, which is currently being validated, utilizes the appearance of abnormal pigmented spots in the fur of the offspring of treated mice to detect mutagens and carcinogens and is dependent on the transplacental transfer of the test compound. K_2CrO_4 (10 and 20 mg/kg) was administered to the pregnant females intraperitoneally, rather than orally, to avoid the conversion of Cr(VI) to Cr(III) by gastric juices. Both doses produced pigmentation in the fur of the offspring, which was interpreted as evidence for the carcinogenicity of Cr(VI) compounds.

Lane and Mass (1977) found that 2.5 mg of chromium carbonyl (Cr(VI)) acted synergistically with 2.5 mg benzo(a)pyrene (BP) to produce carcinomas in syngeneic tracheal grafts in female Wistar-Lewis rats (weight 100 to 150 g). Ten percent of the chromium carbonyl-treated grafts and 36% of the BP-treated grafts developed tumors; there were no metastases following either of these treatments alone. When the chromium carbonyl and BP were combined, however, 42% of the tracheal grafts produced tumors and 30% of these metastasized to the lung.

Although the data indicate the carcinogenic potential of certain Cr(VI) compounds when experimentally administered to laboratory animals, they have little relevance to exposure under environmental conditions because of the route of administration and the large doses used. There were no indications in the literature of any carcinogenic effect resulting from chromium compounds in drinking water.

C. Summary

Trivalent chromium (2.5 to 5.0 μg/kg) has beneficial effects when given to animals with chromium deficiencies. In rats, chromium enhances glucose uptake, decreases circulating cholesterol levels without affecting the levels of aortic lipids, decreases aortic plaques and increases the life span of the animal.

In rats, the LD_{50} for intravenously administered Cr(III) is 10 mg/kg. Acute toxicity studies indicate that a variety of structures (tracheal epithelium, kidney and testis) are sensitive to large doses of chromium. Continuous inhalation exposure of male Sprague-Dawley rats to sodium chromate (Cr(VI)) at 0.173 $mmol/m^3$ of air (59 mg/m^3) caused extensive damage to the tracheal epithelium and submucosal glands within 24 hours. In female mongrel dogs weighing 8 to 16 kg, injection of 1.25 mg potassium dichromate (Cr(VI)) (0.16 to 0.5 mg/kg) into the renal artery caused preferential damage to the first third of the proximal convoluted tubule within 72 hours. Intraperitoneal administration of massive doses (10 to 20 mg/kg body weight) of sodium dichromate (Cr(VI)) also produced changes in the proximal convoluted tubule of the kidney.

In subchronic experiments, intraperitoneal administration of either Cr(III) or Cr(VI), at average total doses of 63 and 126 mg (2 mg/kg body weight/day), produced congestion and tissue degeneration in the brain, kidney and myocardium of rabbits. In another rabbit study, intraperitoneal exposure to Cr(III) and Cr(VI) compounds caused a degeneration of the seminiferous epithelium of the testis and the disappearance of spermatocytes.

Based on the chronic toxicity studies available in the literature, there do not appear to be any detrimental effects from long-term ingestion of low levels of chromium in drinking water. The maximum nontoxic level of K_2CrO_4 (Cr(VI)) in drinking water was 500 ppm (mg/L) (83 mg/kg) for mature white rats. Sprague-Dawley albino rats given water containing 25 ppm (mg/L) (i.e., 1.87 and 2.41 mg/kg/day of either Cr(III) or Cr(VI) for male and female rats, respectively) of either chromium chloride (Cr(III)) or potassium chromate (Cr(VI)) for one year exhibited no pathological effects. Female dogs given 0.45 to 11.2 mg/L of K_2CrO_4 (Cr(VI)) (0.012 to 0.30 mg/kg/day) in their drinking water for four years showed no abnormalities in physical condition, food consumption, or growth rate.

The limited evidence available suggests that Cr(III) is not teratogenic. No conclusion is possible at this time as to whether Cr(VI) is teratogenic under environmental conditions of exposure. The LD_{50} for Cr(VI) injected into four-day-old chick embryos is approximately 0.06 mg/egg, compared to the LD_{50} estimated for Cr(III) of 0.80 mg/egg.

Many mammalian and microbial mutagenicity studies indicate that Cr(VI) compounds are mutagenic. On balance, the evidence suggests that exogenously administered Cr(III) is not mutagenic. Cr(VI) compounds (sodium dichromate, chromic acid, calcium chromate, and potassium dichromate) consistently produced increases in His^+ revertants in Salmonella typhimurium at concentrations of 10 to 200 μg/plate, whereas Cr(III) compounds (chromium potassium sulfate and chromium chloride) did not. The mutagenicity of Cr(VI) can be inhibited by the introduction of reducing agents into the system. However, both chromium chloride (Cr(III)) (0.4 μg/mL) and potassium dichromate (Cr(VI)) (0.1 μg/mL), when added separately to tertiary fetal mouse cell cultures, produced chromosomal aberrations but few sister chromatid exchanges.

The most common carcinogenic response to the administration of Cr(VI) compounds has been injection-site sarcomas. When pellets of Cr(VI) compounds are implanted in the passageways of the lungs, carcinomas are induced. Tumors, however, are not induced in animals exposed to either Cr(VI) or Cr(III) compounds by inhalation or ingestion from the diet.

VI. HEALTH EFFECTS IN HUMANS

In humans, small amounts of Cr(III) are required for normal glucose metabolism. Some forms of chromium may also be important in the metabolism of lipids and other carbohydrates.

The toxicity of chromium has been attributed primarily to Cr(VI), which has been shown to produce liver and kidney damage, internal hemorrhage, dermatitis and respiratory problems and, in addition, is a suspected carcinogen, especially in the occupational setting. Doses of 0.5 to 1.5 g of $K_2Cr_2O_7$ have been fatal in humans and the estimated oral LDL_0 in children for $K_2Cr_2O_7$ is 26 mg/kg (9.2 mg Cr(VI)/kg) (NIOSH 1983).

A. Beneficial Effects

Impaired glucose tolerance was restored to normal in four out of ten elderly subjects after supplementation of their diet with tablets containing 256 µg chromium chloride (3.66 µg/kg based on 70-kg man (ICRP 1975)) or 50 µg Cr(III) (0.7 µg/kg based on 70-kg man (ICRP 1975)) administered three times a day with meals. Prior to supplementation, there was no marked difference between the mean fasting serum chromium levels of young (control group) and elderly groups of subjects (i.e., 23.2 ± 1 and 21.7 ± 3 ng chromium/mL, respectively) (Levine et al. 1968). It was therefore apparent that serum chromium levels did not reflect total body chromium stores.

Administration of 500 µg of chromium/day (7.1 µg/kg/day based on a 70-kg man (ICRP 1975)) for 60 days in a readily absorbable form (sodium chromate reduced by ascorbic acid) to 12 maturity-onset diabetics markedly improved blood glucose tolerance and reduced serum insulin levels (Nath et al. 1979).

Gurson and Saner (1971) have studied protein-energy malnutrition syndrome in infants and its relationship to chromium levels in the body. In 9 out of 14 cases of marasmus (mean age 15 months), the rate of glucose removal from the blood in an intravenous glucose tolerance test improved significantly ($P<0.01$) 16 hours after administration of a single dose of 250 µg of chromium chloride (Cr(III)) (0.027 mg/kg based on a 9-kg baby (USPHS 1976)). When the glucose tolerance test was repeated after 8 to 40 days of hospitalization, the glucose removal rate of the nine infants was still significantly higher than the initial rate ($P<0.001$). However, administration of a second dose (250 µg or 0.027 mg/kg based on a 9-kg baby (USPHS 1976)) of chromium chloride at this time produced no change in the glucose removal rate. No changes were observed in five infants. The authors concluded that chromium was the factor responsible for correction of the abnormal glucose removal rate in approximately two thirds of the protein-energy malnutrition cases.

In a preliminary study, Saner (1979) investigated chromium metabolism in six children from two families with early coronary heart disease. The children were subjected to an oral glucose tolerance test and their urine chromium/creatinine ratios were examined. The glucose tolerance curve for three of the six children exhibited glucose values greater than 120 mg/100 mL two hours after glucose loading. Moderate increases in the chromium/creatinine ratios were only noted, however, in three of the six children. In addition, the mean values of the ratios before and after loading remained statistically unchanged (t_{dep} = 0.7771, NS). This contrasts with the expected and consistent increase in ratios in normal subjects following glucose loading. The initial ratios in the children were, however, higher than in normal age-matched controls ($P<0.001$). These findings indicated a defect in chromium metabolism which Saner (1979) speculated could precede atherosclerosis in the children and which might improve with prophylactic treatment with GTF. Hence, chromium may play a role in the metabolism of some lipids and carboydrates.

Freund et al. (1979) studied the effect of Cr(III) in a 45-yr-old female patient who was on total parenteral nutrition for five months and had developed severe glucose intolerance, weight loss, and a metabolic "encephalopathy-like" state of confusion. Supplementation of her intake with 150 μg of chromium chloride (Cr(III))/day (3.0 μg/kg based on the patient's average weight of 50 kg) reversed the glucose intolerance, reduced the insulin requirement, and resulted in weight gain and the disappearance of encephalopathy.

It is noteworthy that an estimated adequate and safe intake for chromium of 50 to 200 μg/day for adults has been established (NAS 1980a,b). This range is based on the absence of signs of chromium deficiency in the major part of the United States population consuming, on the average, 60 μg of chromium per day. Estimated daily adequate and safe intakes for younger age groups are derived by extrapolation on the basis of expected food intake as follows: 0.01 to 0.04 mg chromium for infants less than six months of age; 0.02 to 0.06 mg for infants between six and twelve months; 0.02 to 0.08 mg for children between the ages of one and three years; 0.03 to 0.12 mg for four- to six-year-old children; 0.05 to 0.2 mg for children from seven years to adulthood (NAS 1980b).

B. Toxic Effects

1. Acute Toxicity

Acute systemic poisoning from a single dose of a chromium compound is relatively uncommon. Only 69 cases of accidental or intentional ingestion of potassium bichromate were reported up to 1935 (Goldman and Karotkin 1935). Since then six cases have been reported in the available literature and these are discussed below. The clinical course of a patient after potassium bichromate (Cr(VI)) intoxication is often complex. The immediate symptoms are generally nausea, repeated vomiting, and diarrhea. These may be followed by hepatic and/or renal failure, a decrease in the number of blood platelets, and internal bleeding. The central nervous system may be affected either directly or indirectly because of hepatic or renal failure. As little as 0.5 to 1 g of potassium bichromate is potentially lethal (based on accidental and intentional ingestion of potassium bichromate) (Moeschlin 1965), and acute poisoning is often fatal, regardless of the treatment available.

Goldman and Karotkin (1935) reported on a 25-year-old female who was admitted to hospital two days after the ingestion of a heaping teaspoonful of potassium bichromate dissolved in water (concentration not stated). The woman complained of dizziness, intense thirst, vague abdominal pains and vomiting, but denied having taken any medication before admission. She was uncooperative and refused routine blood chemistry. Vomiting continued on a daily basis for 18 days. Pain and tenderness in the liver region, indicating severe hepatitis, were reported on the fourth and fifth days. On the sixth day, the blood count was 15,000 leukocytes/mm^3 (normal level approximately 3,000 to 7,000/mm^3). The 24-hour urine output was 600 mL with a specific gravity of 1.012. In addition, there was a trace of albumin and a few red and white blood cells. These findings indicated severe nephritis. Nine days after ingestion of the poison, the blood non-protein nitrogen level peaked at 205 mg/dL and the creatinine level was 8 mg/dL. At this point the patient admitted taking potassium dichromate, and chromium was subsequently identified in the urine (no measurements were made on plasma chromium levels). An erythematous, pin-point, macular rash (a nonspecific toxic rash) appeared on the arms and legs (day 9), and spread rapidly to confluency, then faded from day 13 to day 18. Oliguria persisted for 12 days after ingestion. The patient was discharged in good health after about five weeks. Two and one-half months later when reexamined, her blood pressure and urine were normal (Goldman and Karotkin 1935).

Two cases of accidental poisoning from oatmeal contaminated with 0.25% potassium bichromate (Cr(VI)) (exact amount ingested unknown) produced severe abdominal pain and vomiting within ten minutes, followed by diarrhea and a blue tint to the skin (Partington 1950). Both patients recovered shortly after the incident (no other details were given).

Kaufman et al. (1970) reported the fatal poisoning of a 14-year-old boy who ingested about 1.5 g of potassium dichromate (Cr(VI)) (0.03 g/kg based on a 50-kg 14-year-old boy (Altman and Dittmer 1974)). Ingestion was followed immediately by nausea and vomiting. Emergency treatment included gastric lavage, and an analysis of the aspirate confirmed the presence of chromate in the stomach. The boy was admitted to the hospital and given parenteral fluids in an attempt to induce diuresis. Twenty-four hours after admission he became anuric, and the blood urea nitrogen level rose to 67 mg/dL. After three days he was transferred to another hospital where peritoneal dialysis was continuous with a 2 L/exchange for a total of 126 L over an 84-hr period. His white blood cell count was 22,000/cm^3. Hematemesis with hypotension occurred. Four days after ingestion, the patient received plasma, whole blood, and antacids. He became progressively icteric and agitated and finally became comatose on the sixth day. The blood ammonia level at that time was 342 μg/100 mL. He was given milk of magnesia, enemas, and neomycin. Peritoneal dialysis was discontinued with no response noted in the patient and no urine output. Serum chromium decreased from an initial value of 590 μg/100 mL to 30 μg/100 mL. On the ninth day the patient went into convulsions and a tracheostomy was performed; because of hyperkalemia, peritoneal dialysis was reinstituted for a total of twelve, 2 L exchanges. His condition deteriorated and on day 11 he died. An autopsy revealed significant changes in the liver, kidneys, brain and lungs. The liver was congested, showing diffuse necrosis, hemorrhage and destruction of the lobules, whereas the portal areas were normal. The kidneys were pale and enlarged, with necrosis of the convoluted tubules; the glomeruli were normal. The lungs were congested and there was a pleural effusion; the brain showed marked enlargement with edema. Tissue chromium levels in mg/100 g were as follows: liver, 2.94 (normal 0.016); kidneys, average 0.73, (normal 0.06); and brain, 0.06 (normal 0.002).

Fristedt et al. (1965) reported a case of survival after accidental ingestion of an estimated 15 g of chromic acid (Cr(VI)) as plating fluid. Vomiting began about five minutes after ingestion and persisted for 24 hours with frequent attacks of diarrhea occurring for five days. Anuria, indicating acute renal failure, lasted for 16 days, and dialysis was performed on days 7, 10 and 12. Anemia developed and became increasingly severe with a minimum erythrocyte count of $1.6 \times 10^6/\text{mm}^3$ on day 33. Recovery was apparently complete within about 13 weeks.

Sharma et al. (1978) reported the successful treatment of an 18-year-old girl who had ingested "a few grams" of potassium dichromate (exact amount unknown). Initial symptoms were nausea and repeated vomiting, followed three hours later by diarrhea. Five hours after ingestion, gastric lavage was carried out and the presence of potassium dichromate in the aspirate was confirmed. Intravenous fluid and furosemide were given. Hemoglobin was 14 g/dL, blood urea nitrogen 14 mmol/L and the urine contained protein (amount not specified) and a few granular casts. The patient excreted 200 mL of urine in the next 24 hours, developed jaundice, and reported tenderness in the epigastric (liver) region. Oliguria continued beyond four days, at which time the patient was transferred to another hospital. At that time, her hemoglobin had dropped to 7 g/dL and blood urea nitrogen had increased to 45 mmol/L. Eight days after ingestion, the patient was dialyzed; four days later, she passed 800 mL of urine, indicating partial recovery of renal function. A renal biopsy on the 18th day showed a loss of tubular epithelium, interstitial edema, and evidence of regeneration, all indicating acute tubular necrosis. A liver biopsy showed no abnormality. Recovery was eventually complete with no residual renal or hepatic abnormality.

2. Subchronic and Chronic Toxicity

The subchronic and chronic effects of chromium are treated together, with information derived almost entirely from occupational exposures of several years' duration. Effects have been observed on the respiratory system and skin.

Davids and Lieber (1951) have provided the only available study on human health effects of chromium in drinking water. A family of four persons on Long Island, drinking for three years from a private well containing approximately 1 mg Cr(VI)/L (0.03 mg/kg/day for 70 kg human), suffered no adverse health effects detectable by physical examination (no further details were given).

a. Respiratory Effects

Bloomfield and Blum (1928) examined 23 individuals (19 exposed and 4 non-exposed controls) who had worked from one week to three years in chromium plating industries. Daily exposure ranged from 0.1 to 5.6 mg of chromic acid (Cr(VI)) per m^3 of air. Among the 19 exposed workers, 16% had a perforated nasal septum, 21% had an ulcerated septum, 47% had marked inflammation of the mucosa, 42% had slight inflammation of the mucosa and 58% had frequent nose bleeds. Most of the affected workers had more than one symptom. The authors concluded that daily exposure to chromic acid concentrations exceeding 0.1 mg/m^3 was likely to cause injury to nasal tissues; they did not, however, determine whether the effects resulted from exposure to the acid or to chromium alone.

Mancuso (1951) observed nasal septum perforations in 63% of 97 chromate plant workers exposed to both Cr(III) and Cr(VI) in concentrations as high as 1 mg/m^3 (period of exposure not stated). The reported incidence of rhinitis was 86.6% and that of pharyngitis was 42.3%.

In a survey by the U.S. Public Health Service (USPHS 1953), 509 of 897 chromate workers (57%) were found to have perforations of the nasal septum (period of exposure not stated). Time-weighted exposures for the group ranged from 0.10 to 0.17 mg of water-soluble Cr(VI) per m^3 of air to 1.07 mg of chromium-bearing dusts per m^3 of air. No significant conclusions were reached concerning the relationship of perforated nasal septa to either the duration of exposure or the incidence of lung cancer.

Royle (1975b) reported a comparison of chromium platers in the West Riding of Yorkshire with a closely matched control group of manual workers in the same area. A standard morbidity questionnaire for respiratory symptoms (i.e., chronic bronchitis, asthma, etc.) was used for 997 platers and 1117 controls, but nc physical examinations or physiological tests were performed. In general, more respiratory symptoms were reported in the population of platers. No significant difference (no P value given) was found between the sex, age and tobacco smoking habits of the two groups. However, a higher proportion of platers (651 platers with a total of 5,568 years of exposure to dust compared to 550 controls with a total of 5,136 years) had worked under conditions of high dust exposure (not necessarily chromium dust). A greater number of platers had also worked at a coal mine (114 platers and 54 controls), a foundry (231 platers and 83 controls) and at cotton, flax or hemp mills (95 platers and 32 controls), although more controls had worked in asbestos processing (36 platers and 93 controls). All four differences were statistically significant ($P<0.001$). When both chromium platers and control populations were divided into "dust-exposed" and "non-dust-exposed" groups, the disparate symptomatology was more marked in the dust-exposed subjects, regardless of whether they were platers or control subjects. Thus, the increase in respiratory problems may be related to dust exposure in general and not to exposure to chromium dust. The chromic acid air content measured in 42 plants was less than 0.03 mg/m^3 in all but two cases. The two exceptions were large plants where the chromic acid air content was greater than 0.1 mg/m^3. The chromic acid content of the dust ranged from 0.3 to 97 mg Cr(VI)/g in 35 of the 42 plants. Higher ranges of up to 298 mg Cr(VI)/g were found in four plants, whereas three plants had an upper limit of only 3.9 mg Cr(VI)/g.

Dynamic spirometric measurements were found to be lower among workers employed in the chromium industry. Bovet et al. (1977) tested 44 men from 17 chromium electroplating plants in Switzerland for their vital capacities, forced expiratory volume in one second, and forced expiratory flow at 25% to 75% of the forced expiratory volume. The 44 men were divided into three groups according to the chromium/creatinine ratio found in their urine; the ratio was used as an estimate of the absorption of the metal. The low group had a ratio of 6.0 μg chromium/g creatinine or less; the middle group, 6.1 to 15.0 μg chromium/g creatinine; and the high group, a ratio of 15.1 μg chromium/g creatinine or more (chromium content in the air was not stated). Forced expiratory volume and forced expiratory flow were lower among those workers with higher urinary chromium, but no effect of chromium was observed on vital capacity. The three groups did not differ significantly (no P value given) in age, time of exposure, or smoking habits.

Langard (1980) examined a total of 140 male workers in a Norwegian ferrochromium and ferrosilicon plant for possible symptoms of respiratory disease and impaired lung function. The study group included 60 ferrochromium workers, 33 ferrosilicon workers, and 47 maintenance workers whose time was divided fairly equally between the maintenance shops and both groups of furnaces. Each of these workers had been employed for 15 or more years. The atmosphere at the ferrochromium furnaces contained 0.02 to 0.19 mg total chromium/m^3 of air. Of the chromium present, 11% to 33% was water-soluble and assumed to be hexavalent; this assumption may not be justified. A control group of 25 subjects employed in the plant for less than five years was selected. In comparison with controls, forced vital capacity was reduced and the prevalence of general obstructive lung disease was increased in each of the three study groups. Comparisons between study groups were inconclusive and no connection with chromates could be documented. Langard suggested that the adverse effects documented might be due to high levels of total dust and the presence of amorphous silica dust.

b. Dermal Effects

Chromium has been of special concern to dermatologists since 1827 when chrome ulcers were first described by William Cummin in Scotland and since 1901 when Hermanni demonstrated contact dermatitis in response to chromium (Burrows 1978).

In a study reported by Denton et al. (1954), a patient with a strong specific hypersensitivity to potassium dichromate was patch-tested on three occasions with 0.005% potassium dichromate ($K_2Cr_2O_7$) solution and the first filtrate of two cement samples which were adjusted to pH 5.5. The concentration of Cr(VI) in the two filtrate samples in terms of $K_2Cr_2O_7$ were

0.0001% and 0.0004%. The patient repeatedly showed a positive erythematous, edematous, papulovesicular patch-test reaction to each of the test solutions. Patch tests with distilled water were negative. Control subjects did not react to any of the test solutions. The authors concluded that the average soluble Cr(VI) content of the cement would be sufficient to cause and maintain an allergic contact-type dermatitis in this patient.

Royle (1975b) reported that the risk of skin ulceration increased progressively with increasing periods (from one year to more than five years) of chromic acid exposure ($P \leq 0.001$), whereas allergic contact dermatitis showed no significant difference with increasing duration of exposure. Whether the effects were due to the acid or to chromium alone, however, was not established (Table VI-1). Royle (1975b) found that approximately 22% of 997 platers employed in Yorkshire had skin ulcers and 25% had contact dermatitis, although the dermatitis may have been nonoccupational in many cases. Analysis of dust samples showed the chromic acid content to range from undetectable to 298 mg Cr(VI)/g of dust. The mean exposure time for the platers was 5.6 years, compared with 4.6 years for controls.

3. Carcinogenicity

Only one study was found in the available literature which suggested a possible link between ingestion of chromium and stomach cancer. Stocks (1957) analyzed the chromium content of soil samples taken from the gardens of houses in Caernarvonshire, Merioneth, and Cheshire (Great Britain) and compared the

Table VI-1 Incidence of Skin Ulcers and Dermatitis in a Plater Population and in Groups with Short, Medium and Long Exposure

	Total plater population (997)[a]	Chromic acid exposure 1 yr (234)[a]	Chromic acid exposure 1 yr to $\underline{5}$ yr (394)[a]	Chromic acid exposure >5 yr (369)[a]	Significance of trend over exposure period
Skin Ulcers					
Number	217	15	84	118	
%	21.8%	6.4%	21.3%	32.0%	0.1%
Dermatitis					
Number	245	56	102	87	
%	24.6%	23.9%	26.0%	23.6%	Not Significant

[a] Number of platers.

Adapted from Royle (1975b).

levels with the incidence of cancer in the household (Table VI-2). The median soil chromium content (0.31 mg/L) for stomach cancer cases was significantly higher (no P value given) than that of the non-cancer control group (0.21 mg/L), and also exceeded the value of 0.25 mg/L for other forms of cancer, although not significantly (no P value given). The percentage of dried soil samples containing 0.3 ppm chromium or more was 52% for stomach cancer, 44% for other forms of cancer, 19% for non-cancers, and 36% for the other controls. Unfortunately, Stocks did not report levels of significance and used median values instead of arithmetic means. This could have biased the degree of correlation. In addition, there was no indication whether the chromium present was actually ingested from food grown in these soils, and, if so how much.

Chronic exposure to chromate dust results in an increased incidence of lung cancer in humans. It is difficult, however, to establish a direct relationship between the level of chromium exposure and respiratory cancer by epidemiological studies. Because there is usually a long period of exposure and a long latent period, the extent and type of exposure recorded at the time of the diagnosis is not necessarily a reflection of the level of exposure encountered initially. Furthermore, since lung cancer has a multiplicity of causes, a particular case may not readily be attributed to chromium exposure alone.

Baetjer (1950) reviewed the literature from Germany, the United States, Switzerland and England regarding the role of chromium in lung cancer. In

Table VI-2 Soil Chromium Levels for Persons Dying With and Without Cancer in Caernarvonshire, Merioneth and Cheshire According to Years of Residence in House

Chromium levels in dried soil (ppm)	Non-cancer 20+[a]	Cancer		
		Stomach 15+[a]	Other 15+[a]	All 0 to 1[a]
0		3	1	
0.1	14	6	7	7
0.2	12	10	7	9
0.3	3	11	7	5
0.4	1	2	3	2
0.5		3	1	1
0.6		1		1
0.7	2	2		
0.8		1		
0.9		1	1	
1.0				1
Total	32	40	27	26
Median	0.21	0.31	0.25	0.25

[a] Years of residence in same house.

Adapted from Stocks (1957).

factories producing chromate and in chromate pigment plants, a total of 120 cases of lung cancer were reported. No morbidity or mortality rates were available for the cases in Germany, but statistical studies in the United States indicated that the rates for cancer of the respiratory tract were significantly higher in the chromate-producing industry than would be expected on the basis of comparable groups not exposed to chromates. A study by Machle and Gregorius (1948), included in the review by Baetjer (1950), reported 42 deaths from respiratory cancer found in five chromate-producing plants in the United States between 1937 and 1947. Mortality data were obtained from records of the group life insurance policies of chromate companies and were compared with similar data from industrial policyholders of the Metropolitan Life Insurance Company for other industrial groups. Analysis showed that 21.8% of all deaths reported in the chromate-producing plants were due to cancer of the respiratory system, compared with 1.4% for the industrial control group. The mean duration of exposure prior to the onset of disease was 17.0 years. Unfortunately, the concentrations of chromates in the air were not known.

Mancuso and Hueper (1951) investigated the incidence of mortality due to cancer in a chromate/dichromate plant between 1931 and 1950 and found seven deaths. Exposure levels were calculated for each of the seven cancer deaths (with adjustments for occupational histories), revealing in each case that the predominant exposure involved water-insoluble chromium compounds. The assumption was here made that insoluble compounds were those of Cr(III); the average exposure then corresponded to 0.32 mg Cr(III)/m^3 of air. The weighted average exposures were adjusted for each change of job performed by the deceased worker during his years at the chromate plant. The average period of exposure to all forms of chromium was 3.33 years. However, the number of cases was too small to provide a statistical correlation with period of exposure. As of 1949, respiratory cancer caused 66.7% of all cancer deaths (six deaths), compared with 11.4% in the controls (male population in Lake County, where the plant was located). The mean latent period was 10.6 years.

Mortality due to lung cancer among 723 English, Welsh and Scottish workmen in chromate-producing plants was followed from 1949 to 1955 by Bidstrup and Case (1956). The process of chromate production involved extraction of chromium from chrome ironstone ($FeO \cdot Cr_2O_3$). Over the six-year follow-up period, there were 12 deaths due to lung cancer instead of the 3.3 expected from the age-specific mortality patterns of the male population of England and Wales, a statistically significant increase ($P<0.005$). Mortality patterns for respiratory cancer in Scotland, however, did not differ significantly from expected values. In addition, social class, place of residence (city versus country) and smoking patterns of chromate workers were not found to differ significantly from those of the main population of England and Wales. No statement was made concerning the concentrations of chromium in the air.

A group of 1,212 chromate workers from three chromate plants (approximately 70% of the total population of chromate workers in 1937) was followed for 24 years by Taylor (1966). Seventy-one deaths due to lung cancer were observed instead of the 8.3 expected from the mortality pattern of U. S.

civilian males. A slight increase in other cancers (34 observed versus 23.9 expected) and respiratory diseases (17 versus 7.8) was noted. Although the chromium concentrations to which the men were exposed were not reported, a positive relationship was found between increased respiratory cancer and cumulative years of experience in the chromate industry.

Langard and Norseth (1975) studied 24 Norwegian workers who had worked for at least three years in a chromate-producing factory between 1948 and 1972. Three case of bronchial cancer were found. Based on data from the Cancer Registry of Norway for a corresponding group of the general population, an observed/expected risk ratio of 38 was calculated. The expected number of lung cancers in the group was calculated by assessing the risk of developing lung cancer for each worker separately for each calendar year of the observation period, using the age-specific incidence rates for cancer as supplied by the Cancer Registry. The total risk for the population of workers was then obtained by adding together the risk for each worker for each year of the observation period. If five years exposure had been taken as the minimum for introduction to the study, the cohort would have consisted of only 14 persons and the observed/expected ratio of lung cancer deaths would then have been 83. All three workers with bronchial carcinoma had an exposure time greater than five years. The exposure was mainly to zinc chromate dust; exposure levels for those who developed bronchial carcinomas ranged from 0.3 to 0.4 mg Cr(VI)/m^3 of air for six to eight years. However, two of the three workers smoked, which may have confounded any causal relationship between chromium and cancer.

Royle (1975a) reported on a retrospective study of chromium platers with three months or more of consecutive employment in 54 chromium-plating plants in the West Riding of Yorkshire. Concurrently, a matched control population, consisting of manual workers with no occupational chromium contact, was defined and subjected to the same trace, interview and mortality search techniques. For the mortality survey, 1,238 chromium platers (1,056 male, 182 female) and 1,284 controls (1,099 male and 185 female, manual workers in plants not exposed to chromium) were available for study. The controls were matched individually to the platers by sex, age, date when last known to be alive and, for current workers, smoking habits. The mortality experience of this population as of May 1972 showed a significant two-fold increase of cancer deaths in the plater population ($P \leq 0.05$). When the deaths occurring before May 1972 were combined with deaths occurring from May 1972 to May 1974 (the first two years of the author's prospective study to follow the remaining defined population), the chromium platers experienced a significant excess of deaths from total malignant diseases: 51 versus 24 for controls ($P<0.01$; if females are excluded, the excess is significant at the $P<0.005$) level (Table VI-3). For male chromium platers, 24 lung cancer deaths were observed for platers versus 13 for controls; however, this difference was not statistically significant. Detailed causes of death are shown in Table VI-3.

Table VI-3 Causes of Death in Combined Retrospective and Prospective Studies to May 31, 1974

	Platers			Controls			Significance of Difference
	Males	Females	Total	Males	Females	Total	
1. Malignant neoplastic disease							
(a) Lung and Pleura (I.C.D.[a] 162-163)	24	0	24	13	0	13	Not significant
(b) Gastrointestinal (I.C.D. 150-159)	8	3	11	4	0	4	Not significant
(c) Other sites, including genital and "primary site not specified" (I.C.D. 140-199, excluding (a) and (b)	12	4	16	5	2	7	Not significant
Total malignant neoplastic disease	44	7	51	22	2	24	1% (excluding females, 0.5%)
2. Primary respiratory disease (non-neoplastic)	23	1	24	18	1	19	Not significant
3. Cardiovascular	55	3	58	46	2	48	Not significant
4. Other causes	8	1	9	10	3	13	Not significant
Total	130	12	142	96	8	104	Not significant

[a] International Classification of Diseases.

Adapted from Royle (1975a).

An industrial hygiene study was carried out in 42 of the 54 plating plants from which the plater population was derived (Royle 1975b). Air samples were taken at breathing zone height; in all but two cases concentrations were less than 0.03 mg Cr(VI)/m^3 of air. Analyses of dust scrapings from various sites at the 42 plants showed chromic acid contents ranging from 0 to 298 mg/g dust.

Davies (1978) studied workers exposed to zinc and lead chromate dust in three British factories. In two factories the workers were exposed to both lead and zinc chromate dust; in the third factory they were exposed only to lead chromate. For workers in the first two factories, but not the third, an excess of deaths from lung cancer was found, suggesting that exposure to zinc chromate dust increases the incidence of lung cancer. This relationship between zinc chromate dust and lung cancer deaths was also suggested by the work of Langard and Norseth (1975). No information was given on the levels of exposure to chromium compounds. However, exposure for as little as one year was found to be associated with significantly increased mortality: the standard mortality ratio (SMR), equal to observed/expected deaths was nine observed/2.7 expected deaths ($P<0.01$) (Davies 1978).

Hayes et al. (1979) studied mortality rates among male chromium plant production employees located in Baltimore, Maryland, who were employed during the period 1945 to 1974. The number of lung cancer deaths among 1,803 hourly wage employees (laborers) was significantly higher than expected when compared with the male population of Baltimore (SMR = 59 observed/29.16 expected deaths, $P<0.01$). A dose-response effect was suggested by an apparent relationship between duration of employment and mortality. Hourly workers employed three years or more had a higher SMR for lung cancer than those employed three months to two years; and the latter group also experienced a higher lung cancer mortality rate than expected. No information on the level of chromium exposure was given, but the workers were exposed to both dichromates and chromic acid.

In an evaluation of the carcinogenicity of chromium compounds used in the aircraft maintenance industry, Dalager et al. (1980) found that the cancer mortality rate among 276 male electroplaters exposed to chromic acid did not exceed that of the mortality pattern for U.S. males in general (10 cancer deaths versus 8.6 expected). Analysis of the 202 deaths among 977 male spray painters who used zinc chromate, however, revealed a significant excess of cancer, particularly of the respiratory tract (proportionate mortality ratio, defined as observed deaths/expected deaths, of $21/11.4 = 1.84$, $P \leq 0.01$). A positive gradient in the ratio for respiratory cancer occurred with increasing length of employment (Table VI-4). Information on cigarette smoking was not available, however, and no estimates of zinc chromate exposure were given.

Langard et al. (1980) conducted a study in Norway to determine if workers exposed to Cr(III) were at an increased risk for developing lung cancer or other forms of cancer. The study included 325 ferrochromium workers, retired or currently employed, who had started working at the plant prior to 1960 and had worked for over one year at the plant; those who died before 1953 were

Table VI-4 Observed and Expected Deaths from Respiratory Cancer, with Proportionate Mortality Ratios (PMR) and Proportionate Cancer Mortality Ratios (PCMR), Among White Male Painters

Duration of employment (Years)	Observed deaths	Expected deaths[a]	PMR[b]	Expected respiratory cancer deaths[c]	PCMR[d]
<5	9	6.4	1.41	7.2	1.25
5 to 9	6	3.0	2.00	4.0	1.50
≥10	6	2.0	3.00[e]	3.2	1.88

[a] Expected values based on age-, time- and cause-specific proportionate mortality for U.S. white males.

[b] PMR = observed deaths/expected deaths.

[c] Expected values based on age-, time- and site-specific proportionate cancer mortality for U.S. white males.

[d] PCMR = observed respiratory cancer deaths/expected repsiratory cancer deaths.

[e] Significant at the P = 0.01 level.

Adapted from Dalager et al. (1980).

excluded from the study population. The Cancer Registry of Norway provided cancer incidence rates for all cancers and for each site for comparison purposes.

Air sampling conducted in 1975 showed an average dust concentration of 10.4 mg dust/m^3 of air from personal samples; these samples contained a mean chromium concentration of 0.093 mg/m^3, with 11% to 33% of the total chromium content being made up of water-soluble chromium (Cr(VI)). The chromium concentrations in the personal air samples ranged from 0.01 to 1.30 mg/m^3 (Langard et al. 1980).

Ratios of observed/expected cases of lung cancer were calculated for the seven observed cases among the 325 ferrochromium workers. A ratio of 7/3.1 = 2.26 ($P<0.08$, one-tailed) resulted when the incidence rate for the total Norwegian male population was used as the expected value. When the ratio was recalculated using the age-corrected incidence of lung cancer for men in the rural region where the plant was located, a value of 3.89 was obtained ($P<0.06$). When the seven cases were compared to the two cases in the nonferrochromium study population from the plant, the lung cancer incidence of the ferrochromium workers was found to be 8.5 times that of the other employees ($P<0.026$, one-tailed).

Langard et al. (1980) stated that these results indicate a causal relationship between occupational exposure of the ferrochromium group and increased incidence of lung cancer. Because perforation of the nasal septum was found in two workers and Cr(VI) was found in personal air samples, it was assumed that these workers had been exposed to significant amounts of Cr(VI) that contributed to the increased incidence in lung cancer. Because the risk ratio for those workers exposed to both Cr(III) and Cr(VI) was lower than the ratios for Cr(VI) exposure in previous studies (e.g., Taylor 1966, Enterline 1974, Langard and Norseth 1975), Langard et al. (1980) concluded that exposure to Cr(III) did not increase the risk over that of exposure to Cr(VI) alone, and that the results of the study did not support the suggestion that Cr(III) exposure entails a lung cancer hazard similar to that of Cr(VI).

In a similar study, Axelsson et al. (1980) examined a cohort of 1,932 workers in a Swedish ferrochromium plant to determine whether occupational exposure was associated with an increase in mortality rates or cancer incidence. The cohort included all men employed at the plant for at least one year during the period from 1930 to 1975. The workers were classified by their working sites within the plant, divided into four exposure groups for Cr(III) and Cr(VI). Exposure to Cr(III) was high for workers at arc-furnaces (2.5 mg of Cr(VI) plus Cr(III)/m^3 of air); the arc-furnace operation also had the highest level of exposure to Cr(VI) (0.25 mg/m^3 of air). Expected death rates and cancer incidences were calculated using age-specific rates from the county in which the plant was located. The total number of cancer deaths was slightly lower than expected (69 observed/76.7 expected) and the number of deaths from respiratory cancer was also less than expected (5 observed/7.2 expected). When the deaths were grouped by work site, maintenance workers were found to have an increased death rate from cancer (18 observed/13.6

expected) as well as an increased incidence of respiratory cancer ($P<0.038$). No increase was found for the heavily exposed arc-furnace workers. The authors concluded that the risk of developing respiratory cancer from exposure to Cr(III) was considerably less than the risk from exposure to Cr(VI).

Axelsson and Rylander (1980) found no increase in lung cancer mortality in a Swedish population exposed to exhaust emissions from nearby ferro-alloy industries. Analysis was based on 810 lung cancer deaths occurring from 1961 to 1975. The age-adjusted mortality rates were compared to those of other communities in the same county with similar population size and to that of Sweden as a whole. A strong relationship between population density and lung cancer mortality was found, but when this factor was controlled, no differences in mortality rates were found between the communities with ferro-alloy industries and the remainder of the county. While no exposure levels were available for the study period or previous years, the air concentration of chromium measured in an air pollution control from 1976 to 1979 ranged from 100 to 400 mg/m^3, which was 50 to 100 times higher than the concentration in a rural area without industrial emissions.

In a Finnish study of 45 patients with malignant tumors of the nose and paranasal sinuses, no relationship between exposure to chromium and these cancers was found (Tola et al. 1980). Cases were reported in the Finnish Cancer Registry from 1970 to 1973 from Southern and Southwestern Finland; the controls were also chosen from the Cancer Registry (excluding respiratory cancer) for the same geographical area. The cases and controls or their next of kin answered a questionnaire about occupation and leisure activities. No specific exposure was significantly over-represented among the cases. There were no reports of exposure to nickel, chromium or welding of stainless steel in either the cases or controls.

Chromate workers in the preceding studies were exposed to various Cr(III) and Cr(VI) compounds as well as to other materials. None of the studies presented conclusive evidence regarding the causative agent of the pulmonary carcinomas observed. In addition, most of the studies did not correct lung cancer rates for cigarette smoking or for exposure to other pulmonary carcinogens. It is apparent from the studies, however, that the increased incidence of lung cancer resulted from long periods of exposure to material present in plants either manufacturing or using chromium compounds. These epidemiological studies of lung cancer in man do not permit either the derivation of meaningful dose-effect relationships or extrapolation of the data to no-effect levels.

Further evidence for the potential carcinogenicity of Cr(VI) compounds in humans is provided by the work of Stella et al. (1982), who determined the frequency of sister chromatid exchange (SCE) in lymphocytes from workers who had been exposed to chromic acid fumes in a plating factory. The mean frequency of SCE per metaphase in 12 assays on 10 subjects was 8.08±2.67, significantly higher ($P<0.001$) than the mean control value of 6.31±1.56. Similar increases in chromosomal aberrations and SCE were reported by Sarto et al. (1982). Both groups of investigators observed a negative correlation between frequency of SCE and the age of the subjects.

C. Summary

Chromium (III) is an essential micronutrient required for normal glucose metabolism and may also be important in the metabolism of lipids and carbohydrates. An estimated adequate and safe intake for chromium of 50 to 200 μg/day for adults has been established. Acute chromium poisoning is rare. Ingestion of gram quantities of Cr(VI) can be fatal, causing liver and kidney damage, as well as thrombocytopenia and internal hemorrhage. However, chronic ingestion of an estimated 1 mg/L of Cr(VI) in drinking water over a three-year period did not produce any adverse health effects in one family studied.

Subchronic and chronic exposure to Cr(VI) in the form of chromic acid can cause contact dermatitis and ulceration of the skin; the risk for the latter increases with increasing periods of exposure. Chronic inhalation of dust or air containing Cr(VI) can cause respiratory problems including perforated or ulcerated nasal septa, decreased spirometric values, and cancer. Recent studies suggest that inhalation of Cr(III) compounds does not pose a significant carcinogenic hazard to humans. However, the association between prolonged inhalation of Cr(VI) compounds and the development of cancer of the respiratory tract in exposed workers has been demonstrated by epidemiological studies; the incidence of lung cancer appears to increase with the period of exposure to Cr(VI).

VII. MECHANISMS OF TOXICITY

At the cellular level, the effects of chromium appear to result from interaction with macromolecules, especially nucleic acids and proteins. This section discusses the mechanism(s) of action of chromium in relation to its biological effects and to toxic effects that include mutagenicity, carcinogenicity, skin sensitization and cellular effects.

A. Biological Effects

Nucleic acids normally contain high concentrations of Cr(III) as well as other metals. Chromium concentrations ranging from 260 to 830 μg Cr(III)/g have been measured in a beef liver fraction consisting of 70% RNA and 30% protein, and concentrations from 50 to 137 μg Cr(III)/g have been found in highly purified RNA fractions (Wacker and Vallee 1959a). The function of chromium and other trace elements in RNA *in vivo* is unknown, although *in vitro* it has been demonstrated that transition elements including chromium stabilize the ordered structure of RNA (Fuwa et al. 1960).

The hyperchromicity of RNA upon heat denaturation is markedly reduced in the presence of transition metals including Cr(III), as evidenced by the reduction of the reproducible maximum absorbancy from 1.26 to 1.08 for tobacco mosaic virus ribonucleic acid (TMV RNA) in the presence of 10^{-4} M Cr(III) (Huff et al. 1964). The transition metals do not, however, protect against the loss of biological activity. For example, Huff et al. (1964) reported that heating TMV RNA in the presence of metal ions resulted in the loss of biological activity due to the hydrolysis of phosphodiester bonds. A decrease in the maximum absorbancy of RNA in the presence of chromium was also reported by Fuwa et al. (1960) (Table VII-1). The tertiary structure of beef liver RNA was stabilized by the transition metals (Cr(III), Mn(II), Ni(II) and Zn(II)), but not by alkali metals (Na(I), K(I) and Li(I)) or alkaline earth-metals (Ca(II) and Mg(II)). Fuwa et al. (1960) suggested that the transition metals, including Cr(III), form intramolecular bonds in the RNA which act to preserve the helical conformation of the molecule. The metal bonds formed may serve as cross-links between nucleotide residues on adjacent turns of the helix, and hence stabilize the hydrogen-bonded secondary structure enclosed within the tertiary loop. The reduction in A_{max} of the melting curve observed by Fuwa et al. (1960) and Huff et al. (1964) supports this hypothesis, since the reduction in A_{max} indicated that complete randomization of the secondary structure was not attained. The site and nature of the intramolecular bonds formed between RNA and Cr(III) and other transition metals, however, remain to be elucidated.

Cr(III) is unique among transition metals in (a) its extremely high concentration in nucleoproteins (Wacker and Vallee 1959b), (b) the stability of its attachment to nucleic acids (Wacker and Vallee 1959b) and (c) the extent of its protection against heat-induced conformational changes in nucleic acids (Fuwa et al. 1960, Huff et al. 1964). However, the significance of chromium's protection against heat-induced conformational changes is not known.

Table VII-1 Effect of Metals on A_{max} of the Melting Curve of Beef Liver RNA at 258 nm

Metal	Concentration (M)	A_{max}[a]	Metal	Concentration (M)	A_{max}
Control[b]		1.22	Li(I)	10^{-3}	1.22
Cr(III)	$3x10^{-4}$	1.14	Na(I)	10^{-1}	1.20
Mn(II)	$3x10^{-4}$	1.17	K(I)	10^{-3}	1.22
Ni(II)	10^{-3}	1.12	Mg(II)	10^{-3}	1.19
An(II)	10^{-3}	1.13	Ca(II)	10^{-3}	1.21

[a] A_{max} values represent single determinations in each case.

[b] 0.025 M NH_4 acetate + 0.025 M NaCl, pH 6.8.

Adapted from Fuwa et al. (1960).

Hexavalent chromium also reacts readily with nucleic acids in vitro and greatly reduces the amounts extractable with trichloroacetic acid by stabilizing nucleic acid bonding (Herrmann and Speck 1954).

The best known interaction of chromium with proteins occurs in the tanning process, which conventionally starts with a solution of Cr(VI). Tanning is initiated by adding a reducing agent to the chromate solution to produce Cr(III) in a polynuclear complex. The tanning reaction is poorly understood but probably involves coordination of chromium to the carboxyl groups of collagen strands, which produces extensive cross-linking (Bowes and Kenten 1948, 1949).

At near physiologic concentrations and a metal-to-protein ratio of 20:1, at pH 5.3, Cr(III) cross-links proteins, as studied with conarachin, a protein isolated from groundnuts (Naismith 1958). Cr(III) can also stabilize polypeptide chains in either random-coil or spiral conformation and it thus prevents thermal transition from one form to the other (Pehelin et al. 1963). Cr(III) has been shown to precipitate proteins from solution; the reaction is reversible and no denaturation occurs (Michael 1939).

In contrast, Cr(VI) reacts with proteins at low pH (pH 4), probably through weak bonds that become weaker at physiologic pH (Grogan and Oppenheimer 1955). In the intact organism, trace amounts of injected or ingested chromium are bound and carried in the blood by the iron-carrying protein siderophilin (transferrin) (Hopkins and Schwarz 1964). The reaction occurs only with Cr(III).

B. Toxic Effects

1. Skin Sensitization

The skin sensitizing effects of chromium appear to result from its interaction with skin proteins. Chromium in the hexavalent form is thought to be reduced to Cr(III) before reacting with the proteins. The sulfhydryl group of cysteine has been proposed as a reductant in skin. Other compounds may also be involved, including methionine, cystine, lysine and histidine (Polak et al. 1973, Samitz and Katz 1964). The toxic effects of chromium in skin include ulceration (Royle 1975b) and contact dermatitis (Denton et al. 1954 Fregert et al. 1969).

There has been considerable speculation on the mechanism of action of chromium on skin. Cohen (1976) postulated that certain metals alter proteins to which they are attached, and that the body recognizes the altered protein rather than the full complex. It is difficult to sensitize the skin with trivalent chromium; and it takes about 20 times more Cr(III) than Cr(VI) for epicutaneous sensitization, and 50 times more for intracutaneous sensitization (Van Neer 1965). Paradoxically, all immunological methods for the detection of sensitization - apart from patch testing and macrophage migration - indicate that Cr(III) is the form involved (Fregert and Rorsman 1964, Cohen 1966, Mali et al. 1966). Polak et al. (1973) have shown that Cr(III) readily complexes with proteins on the skin surface and does not penetrate easily. In contrast, Cr(VI) is not considered to form complexes with organic substances and readily penetrates to the dermis (Mali et al. 1966, Czernielewski et al. 1965). Spruit and Van Neer (1966) reported that Cr(VI) penetrated the skin 10,000 times more readily than Cr(III). Samitz and Katz (1963) suggest that Cr(III), which can form octahedral complexes, has available bonding orbitals to form coordination compounds with the basic nitrogen, oxygen and sulfur atoms of cellular macromolecules. These bonding orbitals are not present in the hexavalent species, which has a tetrahedral configuration.

Samitz and Katz (1964) proposed that before chromium is bound to the skin, it must be reduced to the trivalent state. One gram of skin can reduce an average of 1.06 mg of hexavalent Cr(VI) as dichromate to Cr(III) (mean value calculated from data in Table III-9) (Samitz and Katz 1963, 1964). A mean of 68% of the 1.28 mg Cr(VI) added to approximately 0.8 g of wet skin was reduced to Cr(III) in six cases. Samples from the same piece of skin bound more chromium when treated with Cr(III) (average 2.03 mg Cr(III)/g skin) than with Cr(VI) (average 0.533 mg/g skin) (Tables VII-2 and VII-3). The authors suggested that the skin binds only Cr(III). Hexavalent Cr must therefore be reduced to the trivalent state before becoming bound.

This hypothesis was confirmed by the following experiment. Samples of wet surgical or autopsy skin were freed of fat, weighed into test tubes, then incubated with 50 mg of Cr(VI) as potassium dichromate and approximately 50 µCi of ^{51}Cr in 5 mL at 37° C for 48 hours; the samples were then dialyzed for two weeks to remove unbound chromium. After dialysis the amount of Cr(III) and Cr(VI) bound to the skin were determined by chemical analysis and total

Table VII-2 Covalent Binding of Cr(III) to Breast Skin[a]

Sample Number	Wet skin (g)	Cr found by chemical analysis (mg/g wet skin)	Cr found[b] by radiometric analysis (mg/g wet skin)
1	1.261	1.13	0.890
2	1.037	0.972	0.916
3	0.910	0.790	0.862
4	1.211	1.16	0.837
5	0.950	0.930	0.875
6	0.822	2.46	2.30
7	1.472	0.470	1.05
8	1.315	2.50	2.00
9	0.861	2.58	2.10
10	1.503	2.09	1.96
11	1.111	2.80	2.21
12	1.981	2.24	1.78
13	1.003	2.52	1.75
14	1.319	2.44	2.06
15	0.655	2.46	3.56
16	0.430	2.22	2.03
17	0.697	2.22	2.12
18	0.836	2.18	2.02
19	0.550	2.10	2.29
20	0.632	2.66	2.71
21	0.424	2.58	2.69
22	0.391	2.65	2.84
23	0.406	2.60	2.58
		Mean = 2.03	

[a] All samples were incubated with 50 mg Cr(III).
[b] All values represent single determinations.

Adapted from Samitz and Katz (1963).

Table VII-3 Covalent Binding of Cr(VI) to Skin[a]

Sample Number	Wet skin (g)	Cr(VI) found by chemical analysis (mg/g wet skin)	Cr(III) found by chemical analysis (mg/g wet skin)	Total[b] chromium by radio-activity (mg/g wet skin)
		Abdominal skin		
1	3.010	0.001	0.793	
2	2.665	0.000	0.797	
3	2.290	0.000	0.808	
4	1.856	0.000	0.752	
5	0.890	c	0.819	
6	1.272	c	0.816	
7	1.012	c	0.844	
8	0.816	c	0.792	
		Breast skin		
9	1.259	0.005	0.668	0.706
10	1.659	0.006	0.329	0.351
11	1.029	0.005	0.411	0.442
12	1.313	0.007	0.529	0.586
13	1.534	0.006	0.395	0.435
14	0.826	0.001	0.504	0.570
15	1.210	0.006	0.700	0.697
		Breast skin (subcutaneous fat removed)		
16	1.237		0.541	
17	1.558		0.431	
18	1.302		0.566	
19	1.222		0.570	
20	1.585		0.467	
21	2.104		0.412	
22	1.340		0.650	
23	0.485	0.000	0.622	0.693
24	0.536	0.000	0.430	0.390
25	0.627	0.001	0.489	0.463

continued-

Table VII-3 - continued

Sample Number	Wet skin (g)	Cr(VI) Found by chemical analysis (mg/g wet skin)	Cr(III) found by chemical analysis (mg/g wet skin)	Total[b] chromium by radio-activity (mg/g wet skin)
		Breast skin (subcutaneous fat removed)		
26	0.567	0.001	0.588	0.633
27	0.771	0.000	0.475	0.417
28	0.856	0.001	0.524	0.490
29	0.473	0.000	0.505	0.493
30	0.461	0.000	0.592	0.627
			Mean = 0.533	

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[a] All samples were incubated with 50 mg Cr(VI).

[b] All values are single determinations.

[c] Samples not analyzed because amounts of Cr(VI) found in previous samples were not of analytical significance.

Adapted from Samitz and Katz (1963).

chromium was determined by radioactivity. Hexavalent chromium was virtually undetectable after incubation and dialysis; the bound form of chromium was Cr(III) (Table VII-3). Different types of skin samples exhibit different chromium-binding capacities. Mean values of 0.803 mg/g were reported for abdominal skin and 0.527 mg/g for breast skin. The binding of chromium by subcutaneous fat was negligible.

2. Mutagenicity/Carcinogenicity

The carcinogenic and/or mutagenic effects of chromium may result from its interactions with cellular macromolecules. To better understand these interactions the cellular metabolism of chromium is discussed briefly.

A general scheme for the metabolism of chromium and its binding to cellular macromolecules was given in Figure III-1. Essentially, Cr(VI) traverses biological membranes and is then thought to be reduced to Cr(III) intracellularly (Levis et al. 1977, 1978a; Gruber and Jennettc 1978). After reduction, chromium reacts with nucleophilic cell constituents both of low and high molecular weight. The reduction of Cr(VI) in biological systems can result in transient formation of Cr(V), whose complexes are generally labile and reactive (Jennette 1982). The possibility must therefore be considered that such compounds could represent an ultimate carcinogenic form of chromium.

The coordination binding probably involves nitrogen, oxygen or sulfur ligands. The octahedral complex formed between Cr(III) and macromolecules may take the form of cross-linked bonds either between DNA strands (Jennette 1979) within the RNA molecule (Fuwa et al. 1960, Huff et al. 1964) or between or within protein molecules (Bowes and Kenten 1948, 1949). A possible octahedral configuration for cross-linking of Cr(III) with macromolecules was shown in Figure III-3. Trivalent chromium is postulated to represent the ultimate carcinogen or mutagen (Levis et al. 1977, 1978a); definitive evidence as to whether Cr(III) is the species bound to nucleic acids in mammalian and bacterial studies must await structural studies of the chromium-nucleic acid adducts.

Numerous studies indicate that metal ions interact with nucleic acid strands *in vitro* to influence their base-pairing and conformation (Eichhorn et al. 1971, Murray and Flessel 1976, Shin et al. 1972). If similar reactions take place *in vivo*, they could indeed produce somatic mutations and thus lead to derangements of cellular metabolism and, ultimately, to neoplasia (Goodenough 1978). An alternative effect of chromium might be conformational distortion (base displacement) of double-stranded DNA, which could cause DNA polymerase to stop at, or skip, the distorted site, resulting in a frame-shift mutation (Weinstein 1978).

Recent work by Tsapakos et al. (1981) indicates that DNA cross-links are formed in nuclei from the liver and kidney of rats treated with chromate. Male Sprague-Dawley rats received injections of 0.5 mL of 0.9% NaCl containing various doses of sodium dichromate (Cr(VI)) one hour prior to sacrifice and the preparation of nuclei (Table VII-4). The authors reported a dose-dependent relationship between the amount of sodium dichromate administered and the total number of DNA cross-links. The cross-links in the

Table VII-4 Apparent Frequencies of DNA Lesions Produced in Rat Liver and Kidney Nuclei One Hour After Treatment With Chromate

Tissue	Chromate dose (mg/kg)	Apparent lesion frequency (rad-equiv.)[a]	
		No proteinase	Proteinase
		Cross-links	Cross-links
Liver	20	90±18	-16±9[b]
	50	175±29	-32±16
Kidney	20	40±7	11±7
	50	90±13	14±11

[a] Values were calculated from % DNA remaining on the filter compared to control samples after 9 hr of elution (mean of 3 to 5 experiments) according to the equations described in Ewig and Kohn (1978). Rad-equiv. is the equivalent amount of radiation which would give rise to that number of single-strand breaks. The samples were irradiated to induce random single-strand breaks and to magnify the effects of DNA cross-linking with Cr(III) on molecular size.

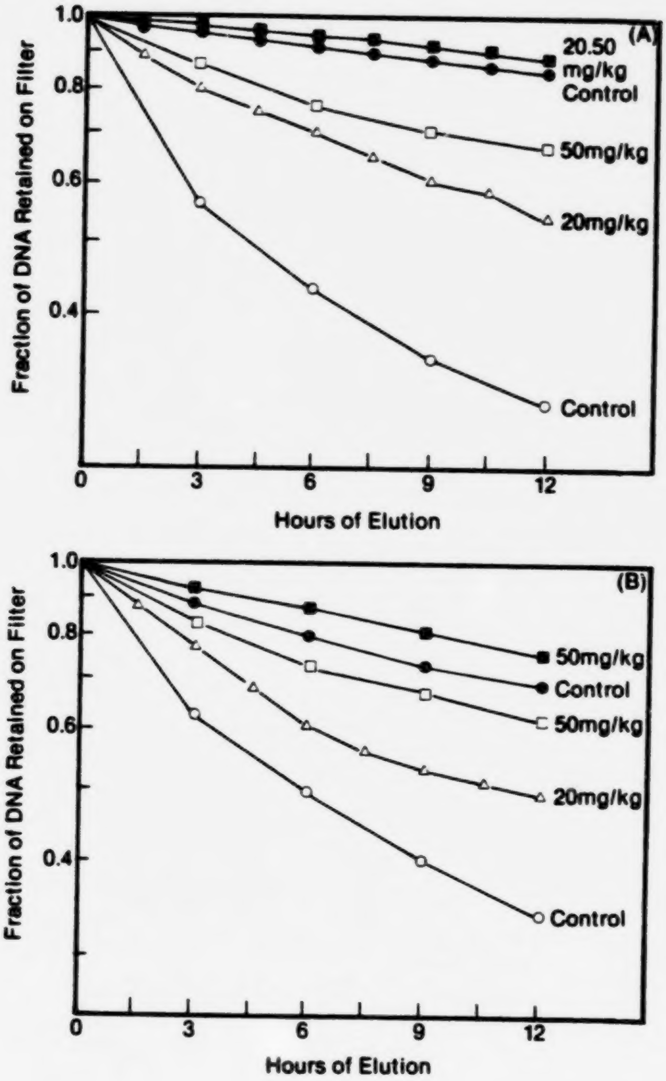
[b] Negative numbers indicate less DNA retained on the filter than control.

Adapted from Tsapakos et al. (1981).

liver and kidney were found to be mostly between DNA and protein, as indicated by their decrease in number after treatment with proteinase. In addition, the results suggested that sodium dichromate induced a small number of interstrand DNA cross-links in kidney, as indicated by the greater number of crosslinks remaining after proteinase treatment in kidney nuclei than in liver nuclei. However, the actual level of chromium present in cross-linked DNA was not reported.

Tsapakos et al. (1981) also reported on the kinetics of alkaline elution of DNA, which appeared to be dependent on DNA strand size. A marked decrease was found in the elution rates of DNA from irradiated nuclei from both liver and kidney taken from dichromate-treated animals (Figure VII-1). The DNA was irradiated to amplify the effects of chromium cross-linking. Radiation induced random, single-strand breakage in untreated DNA and multiple fragments (cross-linked by Cr(III)) in the dichromate-treated samples. Since single-strand breakage is known to increase the elution rate, whereas interstrand DNA and DNA-protein cross-links decrease it (Kohn 1979), the data of Tsapakos et al. (1981) suggest that chromium, probably as Cr(III), does bind to DNA in rats.

Sirover and Loeb (1976) found that both chromium chloride ($CrCl_3$) (Cr(III)) and chromium trioxide (CrO_3) (Cr(VI)) decreased the fidelity of AMV (avian myeloblastosis virus) DNA polymerase for the replication of synthetic polynucleotide templates. Cr(III) at 0.64 mM produced a 3.70-fold change in error frequency and Cr(VI), at 16.0 mM produced a 3.83-fold change. The error frequency was defined as the ratio of incorporation of noncomplementary to



Adapted from Tsapakos et al. (1981). Rats were injected with sodium dichromate (dissolved in 0.9% NaCl) at the indicated doses, then sacrificed 1 hr later. Alkaline elution kinetics were measured either without irradiation (●, ▲, ■) or after exposure to 330 Rads at 0°C (○, △, □).

Figure VII-1 The Effect of Chromate on DNA Elution Kinetics of Rat Liver Nuclei (A) and Kidney Nuclei (B)

complementary deoxynucleotides. If a similar mechanism of action for chromium occurs *in vivo* in mammalian systems, the decreased fidelity of replication could result in somatic mutations or frame-shift mutations.

Tkeshelashvili et al. (1980) reported that both CrO_3 (Cr(VI)) and $CrCl_3$, (Cr(III)) increased the error rate of DNA synthesis by *Escherichia coli* DNA polymerase I on polynucleotide templates in a dose-dependent manner (Table VII-5). Addition of 5 mM CrO_3 (Cr(VI)) increased the error rate approximately 9-fold over controls, whereas 5 mM $CrCl_3$ (Cr(III)) increased the rate 15-fold. Tkeshelashvili et al. (1980) also showed that Cr(VI) increased the frequency of misincorporation of nucleotides by *E. coli* DNA polymerase I on a natural DNA template. The addition of 5 mM Cr(VI) increased the error rate more than fivefold; similar results were also observed for Cr(III).

Zakour et al. (1981) have shown a strong correlation between metals which are unequivocally carcinogenic and/or mutagenic and metals which increase the misincorporation of nucleotides by DNA polymerase *in vitro* using synthetic templates (Table VII-6). Nine of the ten known carcinogens and/or mutagens listed increased the error rate of DNA polymerase by more than 30% at two or more concentrations (Sirover and Loeb 1976, Tkeshelashvili et al. 1979, 1980). This correlation suggests that chromium (Cr(VI) and Cr(III)) mutagenicity may be a result of the effect of the two cations on the fidelity of replication by DNA polymerase.

Table VII-5 Fidelity of DNA Synthesis in the Presence of Chromium[a]

Chromium added (mM)	Complementary nucleotide (dATP and dTTP) incorporation (pmol)	Noncomplementary nucleotide (dGTP) incorporation (pmol)	Error rate[b]
Cr(VI) as CrO_3			
0.0	314	0.0094	1/33,400
0.1	353	0.0122	1/28,900
0.5	386	0.0156	1/24,700
1.0	376	0.0236	1/15,900
5.0	346	0.0969	1/3,570
Cr(III) as $CrCl_3$			
0.0	278	0.0066	1/42,100
0.1	258	0.0093	1/27,700
0.5	242	0.0114	1/21,200
1.0	224	0.0145	1/15,400
3.0	72	0.0130	1/5,540
5.0	14	0.0050	1/2,800

[a] The fidelity of DNA synthesis is a measure of the ability of DNA polymerase to incorporate the correct nucleotides into the strand being synthesized so that the nucleotide is complementary to the one in the template. Poly [d(A-T)] was used as a template. Incorporation in the absence of incubation was 1 pmol dTMP and 0.008 pmol dGMP, which were subtracted from the values in the table. Metal solutions were added to the reactions as indicated, and the assays were preincubated for 15 min at 0°C.

[b] The error rate was defined as the ratio of noncomplementary nucleotide incorporated to total complementary nucleotide incorporated.

Adapted from Tkeshelashvili et al. (1980).

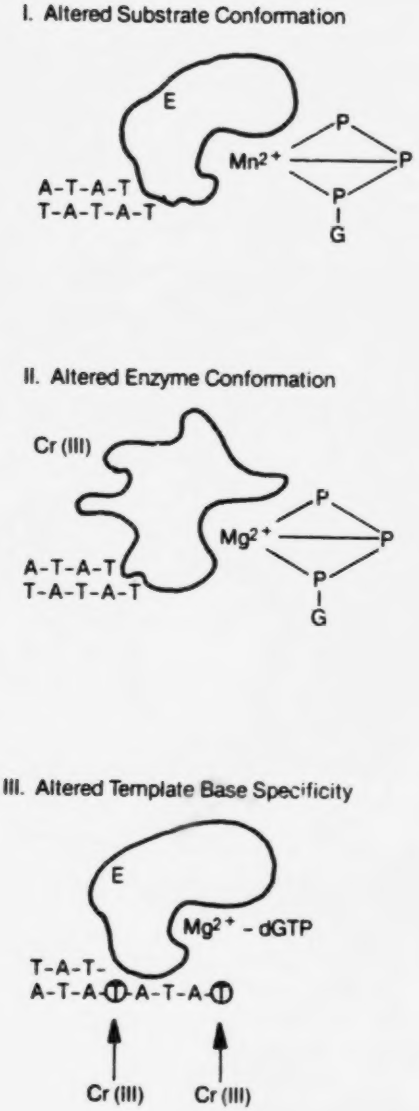
Table VII-6 Correlation of Carcinogenicity and/or Mutagenicity of Metals with Effects on the Fidelity of DNA Synthesis by DNA Polymerase

Metal	Known carcinogens and/or mutagens	Increased misincorporation	No change in fidelity
Ag	+[a]	+	
Al			+
As	+		+
Ba			+
Be	+	+	
Ca			+
Cd	+	+	
Co	+	+	
Cr	+	+	
Cu	+	+	
K			+
Mg			+
Mn	+	+	
Na			+
Ni	+	+	
Pb	+	+	
Rb			+
Se			+
Sr			+
Zn			+

[a] + indicates correlation.

Adapted from Zakour et al. (1981).

Zakour et al. (1981) have suggested three possible mechanisms for the decrease in the fidelity of *in vitro* DNA synthesis by metal ions (Figure VII-2). First, metal ions may alter the substrate conformation by binding to the enzyme. Magnetic resonance studies indicate that the interaction of the metallic ion involves a bridged complex among the enzyme, metal and substrate involving the gamma-phosphoryl group of the substrate (Slater et al. 1972). Zakour et al. (1981) suggest that differences in the conformation of the bound substrate with different metal activators, such as Mg, may explain differences in the fidelity of DNA synthesis. This mechanism, however, would apply only to metals which serve as activators of DNA polymerases; Cr(III) and Cr(VI) are not generally considered to be activators of DNA polymerases. Second, metal ions may also alter the conformation of DNA polymerase by binding to ancillary binding sites on the enzyme. The decrease in fidelity at inhibiting concentrations of metal activators suggests that the metal is binding at sites in addition to the active site. Nuclear magnetic resonance studies have detected ancillary binding sites for Mn (II) on *E. coli* DNA polymerase I (Slater et al. 1972). Reports that non-activating metal cations, including Cr(III) and Cr(VI), decrease the fidelity of DNA polymerase, are compatible with the alteration of DNA polymerase conformation. Zakour et al. (1981) suggest that interactions of metal cations or metal-nucleotide complexes at allosteric sites on the enzyme could change the conformation of the polymerase and enhance misincorporation of nucleotides. Third, metal ions may interact directly with phosphate groups and bases on polynucleotides and thus interfere with complementary base-pairing (Eichhorn and Shin 1968). Cr(III) has been shown to bind to nucleic acids, affecting the fidelity of synthesis by DNA polymerases.


I. Altered Substrate Conformation
E
Mn2+
A-T-A-T
T-A-T-A-T
P
P
P
G
II. Altered Enzyme Conformation
Cr (III)
Mg2+
A-T-A-T
T-A-T-A-T
P
P
P
G
III. Altered Template Base Specificity
E
Mg2+ - dGTP
T-A-T-
A-T-A-T-A-T-A-T
Cr (III)
Cr (III)

Modified from Zakour et al. (1981).

Figure VII-2 Possible Mechanisms for Metal-Induced Infidelity

Zakour et al. (1981) suggest that metal-induced mutations may occur by the interaction of metal cations with DNA polymerase or the DNA template. They further argue that by studying the fidelity of DNA synthesis by DNA polymerases, one is investigating *in vitro* mutagenesis. The correlation of carcinogenicity and/or mutagenicity of metals with alterations of *in vitro* fidelity of synthesis by DNA polymerase is in agreement with this hypothesis (see Table VII-6). However, considerably more experimental evidence is necessary to establish a cause-and-effect relationship between the alteration of fidelity and mutagenicity.

Petrilli and De Flora (1977) have studied the mutagenicity of the Cr(VI) compounds (sodium dichromate, chromic trioxide, calcium chromate, and potassium chromate) and Cr(III) compounds (potassium chromic sulfate and chromic chloride) in the Ames assay using histidine auxotrophic strains of *S. typhimurium* and a mammalian activation system. With Cr(VI), there was a significant increase in His^+ revertant colonies at doses ranging from 10 to 200 μg per plate; Cr(III), however, was not mutagenic, with or without microsomal activation. All hexavalent chromium compounds tested showed complete inhibition of bacterial growth at doses of 400 to 800 μg per plate. Cr(III) compounds, however, did not show toxicity even at 20 mg/plate. The results suggested that Cr(VI) reacted directly with bacterial DNA to produce both frame-shift and base-pair substitution mutations. The latter mutations were amplified by the error-prone recombinational repair of the altered DNA structure.

Petrilli and De Flora (1978b) investigated the mechanism of the metabolic deactivation of Cr(VI) mutagenicity. Hexavalent chromium compounds (potassium chromate, sodium dichromate, chromic acid, basic zinc chromate and basic lead chromate) were found to be mutagenic for His^+ strains of S. typhimurium, inducing both frame shifts and base-pair substitutions. The addition of either microsomal fractions from rat liver or human erythrocyte lysates resulted in a complete loss of mutagenicity. Loss of mutagenicity appeared to be the result of reduction of the metal to the trivalent form. Table VII-7 shows that reducing agents such as ascorbic acid and sodium sulfite prevented hexavalent chromium mutagenicity, in the absence of a microsomal deactivating system, at 100 and 1,000 μg per plate, respectively. The metabolic deactivation by liver microsomal preparations was not affected by these reducing agents. Treatment with an oxidizing agent (potassium permanganate, $KMnO_4$) resulted in enhanced mutagenic effects, probably by maintaining chromium in the Cr(VI) state. $KMnO_4$ also inhibited, in a dose-related manner, the decrease in mutagenicity normally seen with a rat liver microsomal preparations (Table VII-7).

Similar results for the mutagenicity of Cr(III) and Cr(VI) were obtained by Gentile et al. (1981) using the rec-assay. Petrilli and De Flora (1978b) suggested that the apparent lack of mutagenicity of Cr(III) was due to the formation of chemical complexes or insoluble polynuclear species with extracellular components before Cr(III) was able to penetrate the cellular membrane.

Table VII-7 Effects of Reducing and Oxidizing Agents on the Mutagenicity of Sodium Dichromate (Cr(VI))

Treatment	Concentration (μg/plate)	Number of TA 100 revertants: Control	Number of TA 100 revertants: $Na_2Cr_2O_7$[a]	Number of TA 100 revertants: $Na_2Cr_2O_7$[a] + Liver S-9[b]
Controls	0	236±25.9[c]	960±41.5	185±21.0
Ascorbic acid	100	247	238	181
	25	239	890	195
	6.25	220	1,050	183
Sodium sulfite	1,000	198	191	179
	250	225	780	185
	62.5	241	995	168
Potassium permanganate	100	245	1,330	1,170
	25	191	1,090	465
	6.25	205	1,120	168

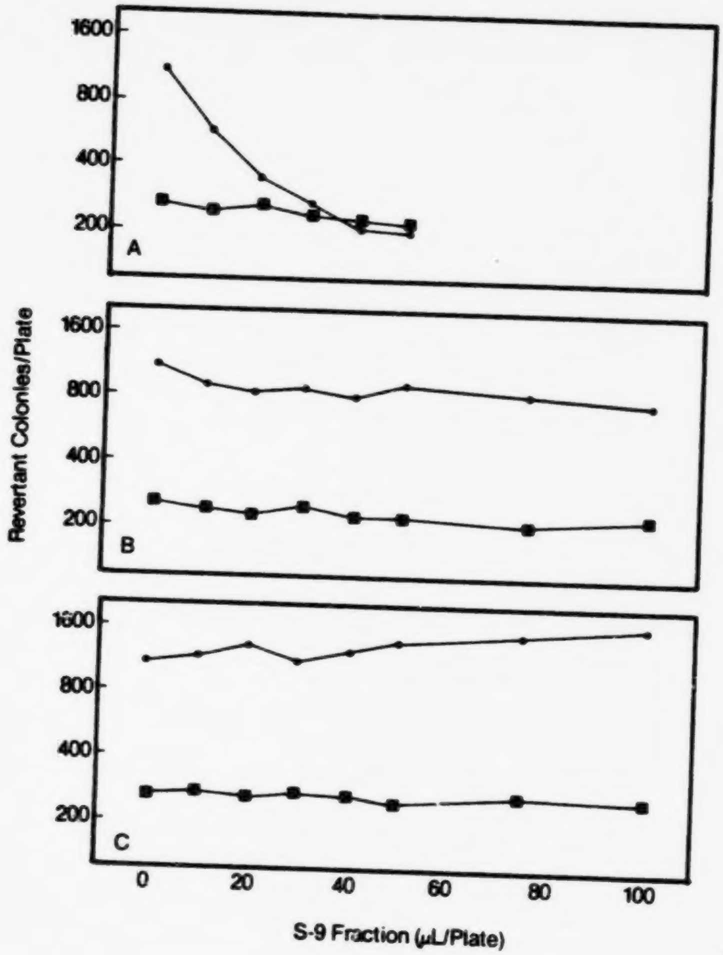
[a] 52 μg/plate.
[b] 50 μL/plate in 0.5 mL S-9 mix.
[c] ± standard error of the mean.

Adapted from Petrilli and De Flora (1978b).

Petrilli and De Flora (1978b) also found that S-9 fractions (the 9,000 x g supernatant fraction of the tissue homogenate used as the microsomal preparation) from rat lung and rat muscle did not deactivate Cr(VI) mutagenicity, whereas an S-9 fraction from rat liver did (Figure VII-3).

The lack of a deactivating mechanism in lung and muscle may explain in part the localization of chromium-induced cancer in human lung (IARC 1973) and the development of tumors in rodents only at implant sites, especially in the respiratory tract and in muscle (Hueper and Payne 1962, Roe and Carter 1969). Possible explanations for lack of deactivation include (a) the absence of an enzyme system for Cr(VI) reduction in lung and muscle, (b) the presence of an oxidizing agent in lung and muscle preparations that may be absent in rat liver or (c) the presence of an inhibitory species in lung and muscle preparations. Further work with highly purified enzyme preparations from these tissues may elucidate the mechanism(s) of metabolic deactivation of Cr(VI) mutagenicity in *S. typhimurium*.

Even though Cr(VI) is readily reduced to Cr(III), as indicated by a decreased incidence in His^+ revertants in the presence of a rat liver microsomal preparation, the converse reaction (i.e., oxidation of Cr(III) to Cr(VI)) could not be demonstrated in the presence of any of the microsomal preparations tested, mitochondrial preparations, human serum, human plasma or human erythrocyte lysate (Table VII-8) (Petrilli and De Flora 1978a). An increase in the number of His^+ revertants, indicating oxidation of Cr(III)



Adapted from Petrilli and De Flora (1978b). S-9 fractions were mixed with 0.5 mL of S-9 mix cofactors.

Figure VII-3 Effect of Microsomal (S-9) Fractions from Rat Liver (A), Lung (B) and Muscle (C) on the Number of S. typhimurium (strain TA 100) Spontaneous Revertants (■) and Revertants Induced by 80 μg of CrO_3^{2-} (●)

Table VII-8 Number of TA 100 Revertants Yielded by Chromium Potassium Sulfate in Preparations from Rat Tissues or Human Blood Components

Metabolic system	His$^+$ revertants/plate Control	 Chromium potassium sulfate (1 mg/plate)
None	189±27[a]	176±22
Liver S-9 mix[b]	178	167
Lung S-9 mix[b]	184	180
Muscle S-9 mix[b]	201	195
Muscle mitochrondria[c]	235	214
Muscle mitochrondria + ATP (2 µmol)	195	187
Muscle mitochrondria + ATP (10 µmol)	192	178
Human serum[c]	194	185
Human plasma[c]	181	170
Human erythrocyte lysate[c]	167	158

[a] Mean ± S.E.M.
[b] 50 µL microsomal fraction in 0.5 mL S-9 mix/plate.
[c] 0.1 mL/plate.

Adapted from Petrilli and De Flora (1978a).

to Cr(VI), was only accomplished in the presence of a strong oxidizing agent ($KMnO_4$). These results suggest that oxidation of Cr(III) to Cr(VI) does not occur through the metabolic systems used in short-term assay systems. Petrilli and De Flora (1978a) suggest that the oxidation of Cr(III) to Cr(VI) in the organism should be a relatively slow process occurring only under conditions of significant retention and accumulation of the metal, and then only in tissues with a low reducing potential.

3. Cellular Toxicity

The toxic effects of chromium at the cellular level appear to result from inhibition of DNA, RNA and protein synthesis as well as from direct covalent binding of chromium to enzymes involved in energy production.

Levis et al. (1977, 1978b) studied the in vitro effects of potassium dichromate ($K_2Cr_2O_7$) on DNA synthesis in BHK cells (hamster fibroblasts). DNA polymerase was inhibited by 0.1 mmol $K_2Cr_2O_7$. Inhibition was related to the duration of treatment (one to four hours) and was enhanced when cells were incubated in Hanks' balanced salt solution as opposed to complete growth medium. Levis et al. (1977) suggested that extracellular reduction of Cr(VI) to Cr(III) was greater in complete growth medium than in Hanks' solution; therefore, cells suspended in Hanks' solution were probably exposed to higher concentrations of Cr(VI). Levis et al. (1978b) also investigated the effect of Cr(VI) on the rates of synthesis of DNA, RNA and protein in BHK cells by measuring changes in the uptake of ^3H-labeled thymidine, ^3H-labeled uridine and ^3H-labeled leucine, respectively. Cr(VI) rapidly inhibited DNA, RNA, and protein synthesis (Table VII-9). The inhibition of DNA synthesis was considerably greater than inhibition of either RNA or protein biosynthesis. A one-hour treatment with 10^{-3} M $K_2Cr_2O_7$ resulted in nearly complete inhibition of DNA synthesis (93%), whereas synthesis of RNA and protein was only inhibited approximately 50%.

Levis et al. (1978a) also studied the effects of chromium chloride (Cr(III)) and potassium dichromate (Cr(VI)) on BHK21 cells and HEp2 cells. Cr(VI) as $K_2Cr_2O_7$ inhibited DNA synthesis markedly at 10^{-4} M and 10^{-3} M in both cell lines, whereas Cr(III) as $CrCl_3$ was not inhibitory below 10^{-2} M in BHK cell cultures. On the basis of a comparison of the toxicity of Cr(III) and Cr(VI), Levis et al. (1978a) suggested that Cr(VI) interferes with nucleotide uptake at the plasma membrane and postulated a direct interaction of Cr(III) with nucleophilic sites in DNA.

Chromium may also exert its toxicity at the cellular level by interfering with enzymes involved in cellular metabolism. For example, daily intraperitoneal administration of 2 mg/kg chromium nitrate (Cr(III)) and potassium dichromate (Cr(VI)) to rabbits inhibited the activity of succinic dehydrogenase, adenosine triphosphatase, and acid phosphatase (Table VII-10); this was accompanied by cellular degeneration and the disappearance of spermatocytes from the testis (Behari et al. 1978). The biochemical and histological changes were more marked in animals treated with Cr(III) than in those exposed to Cr(VI); in addition, the inhibition increased as exposure

Table VII-9 Differential Inhibition of DNA, RNA and Protein Synthesis by Potassium Dichromate in BHK Cell Cultures

Potassium dichromate concentration (M)	Duration of treatment (hr)	Inhibition[a] (% of control macromolecular synthesis)		
		DNA	RNA	Proteins
10^{-3}	1	93	52	45
	3	98	70	60
	6	100	88	75
10^{-4}	1	59	30	0
	3	94	38	21
	6	97	56	25
10^{-5}	1	55	2	0
	3	64	21	4
	6	75	16	0

[a] Inhibition of DNA, RNA and protein synthesis was calculated by extrapolation of macromolecular synthesis-inhibition kinetics from Charts 4 and 5 in Levis et al. (1978b). Inhibition is expressed as a percentage of control synthesis values, which were normalized to 100%. Each value represents a single point read from Charts 4 and 5; no statistical analysis was made.

Adapted from Levis et al. (1978b).

Table VII-10 Activity of Enzymes in Testis of Rabbits Treated Intraperitoneally with Either Chromium Nitrate (Cr(III)) or Potassium Dichromate (Cr(VI)) for 3 and 6 weeks at 2 mg/kg/day

Enzymes	Control	Cr(III)				Cr(IV)			
		3 weeks	% change	6 weeks	% change	3 weeks	% change	6 weeks	% change
Succinic dehydrogenasse (nmol of $K_3Fe(CN)_6$ reduced/min/mg protein)	2.23±0.08[a]	1.80±0.20[b]	-19.3	1.74±0.14[c]	-22.0	1.86±0.22[c]	-16.6	1.70±0.011[c]	-23.8
Adenosine triphosphatase (nmol of Pi liberated/min/mg protein)	117.32±2.69	64.76±1.79[d]	-44.8	47.28±1.84[d]	-59.7	114.60±3.62	-2.3	91.26±1.51[d]	-22.2
Acid phosphatase (nmol of phenol liberated/min/mg protein)	15.25±1.16	18.70±0.67	+2.26	11.64±0.58[d]	-23.7	13.34±0.86	-12.5	14.48±1.02	-5.0

[a] Each value represents mean ± S.E. of 8 rabbits in control and 5 in experimental groups.
[b] $P<0.05$, when compared to control as evaluated by Student's t-test.
[c] $P<0.01$, when compared to control as evaluated by Student's t-test.
[d] $P<0.001$, when compared to control as evaluated by Student's t-test.

Adapted from Behari et al. (1978).

increased from three to six weeks. At six weeks, inhibition of succinic dehydrogenase was nearly the same for Cr(III) and Cr(VI) whereas inhibition of adenosine triphosphatase was nearly three times greater for Cr(III) and inhibition of acid phosphatase was four times greater with Cr(III). Chromium inhibits succinic dehydrogenase and adenosine triphosphatase in testis as do other industrial metals such as cadmium, lead, mercury and manganese. Both enzymes are involved in the energy metabolism of the cell, so their inhibition could cause toxic effects at the cellular level, such as the degenerative changes observed in the seminiferous epithelium of the rabbits.

C. Summary

Cr(VI) readily traverses biological membranes and is reduced intracellularly to Cr(III) by the cytochrome P-450 system and other cell constituents. After reduction, Cr(III) forms octahedral complexes with cellular macromolecules including nucleic acids and proteins. By interacting with these molecules, chromium may influence base-pairing and the conformation of DNA; this may result in somatic mutations. Alternatively, chromium might cause conformational distortion of DNA resulting in DNA polymerase stopping at or skipping a distorted site, leading to a frame-shift mutation. The interaction with nucleic acids may be responsible for the toxic, mutagenic, and carcinogenic properties of chromium. There is evidence to suggest that Cr(VI) is reduced to Cr(III) in skin and that the trivalent chromium readily reacts with proteins. Chromium-protein complexes may be responsible for chromium toxicity in skin. Chromium may also bind to enzymes and exert its toxic effects by interfering with cellular metabolism; in this regard, Cr(III) appears to be more active than Cr(VI). For example, Cr(III) as chromium nitrate and Cr(VI) as potassium dichromate both inhibit succinic dehydrogenase and adenosine triphosphatase in rabbit testis, as do other industrial metals such as cadmium, lead, mercury and manganese. Both these enzymes are involved in energy metabolism of the cell and their inhibition may cause cellular intoxication. Cr(VI) and Cr(III) have both been shown to decrease the fidelity of DNA synthesis by DNA polymerase *in vitro*. It has been suggested that this alteration of DNA synthesis fidelity by Cr(III) and Cr(VI) may result in mutagenicity and/or carcinogenicity. Cr(VI) appears to be the mutagenic form of chromium in *S. typhimurium*, since reduction of Cr(VI) to Cr(III) by microsomal rat liver preparations or reducing agents decreased mutagenicity. Microsomal preparations from rat lung and rat muscle, however, do not decrease Cr(VI) mutagenicity. The lack of deactivation by these preparations may thus explain the susceptibility of the tissues to the development of tumors following implantation of Cr(VI). The reverse reaction (i.e., oxidation of Cr(III) to Cr(VI)) is not accomplished by any of the rat microsomal, rat mitochondrial or human serum components tested in short-term assay systems; this suggests that oxidation of Cr(III) to Cr(VI) may not readily occur in mammalian systems.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic effects. Chemicals which do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic effects occur, while carcinogens are assumed to act without a threshold.

Quantification of Noncarcinogenic Effects

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI), is calculated. The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects, even if exposure occurs over a lifetime. The RfD is derived from a No-Observed-Adverse-Effect-Level (NOAEL), or Lowest-Observed-Adverse-Effect-Level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s). The RfD is calculated as follows:

$$\text{RfD} = \frac{\text{(NOAEL or LOAEL)}}{\text{Uncertainty Factor(s)}} = \underline{\quad}\ \text{mg/kg bw/day}$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based on professional judgment, taking the entire data base of toxicological effects for the chemical into consideration. In order to ensure that uncertainty factors are selected and applied in a consistent manner, the

Office of Drinking Water (ODW) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS 1980b) as follows:

- An uncertainty factor of 10 is generally used when good chronic, or subchronic human exposure data identifying a NOAEL are available and are supported by good chronic, or subchronic toxicity data in other species.

- An uncertainty factor of 100 is generally used: (1) when good chronic toxicity data identifying a NOAEL are available for one or more animal species (and human data are not available), or (2) when good chronic, or subchronic toxicity data identifying a LOAEL in humans are available.

- An uncertainty factor of up to 1,000 is generally used: (1) when limited or incomplete chronic, or subchronic toxicity data are available, or (2) when good chronic, or subchronic toxicity data identify a LOAEL, but not a NOAEL for one or more animal species are available.

The uncertainty factor used for a specific risk assessment is based principally on scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime study for deriving a RfD, the significance of the adverse effect and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent (DWEL) can be calculated. The DWEL is a medium specific (i.e., drinking water) lifetime exposure, assuming a 100% relative source contribution from that medium, at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$\text{DWEL} = \frac{\text{(RfD) x (Body Weight in kg)}}{\text{Drinking Water Volume in L/day}} = \underline{\quad}\ \text{mg/L}$$

where:

Body weight = assumed to be 70 kg for adult

Drinking water volume = assumed to be 2 liters per day for an adult

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (One-day, Ten-day and Longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using a similar equation to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$\text{HA} = \frac{\text{(NOAEL or LOAEL) x (bw)}}{\text{(UF) x (\underline{\quad} L/day)}} = \underline{\quad}\ \text{mg/L}$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. One-day HA for a 10-kg child ingesting 1 L water per day.
2. Ten-day HA for a 10-kg child ingesting 1 L water per day.
3. Longer-term HA for a 10-kg child ingesting 1 L water per day.
4. Longer-term HA for a 70-kg adult ingesting 2 L water per day.

The One-day HA calculated for a 10-kg child assumes a single acute exposure to the chemical and is generally derived from a study of less than seven days duration. The Ten-day HA assumes a limited exposure period of one to two weeks and is generally derived from a study of less than a 30-day duration. The Longer-term HAs are derived for both a 10-kg child and a 70-kg adult and assume an exposure period of approximately seven years (or 10% of an individual's lifetime). The Longer-term HAs are generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

Quantification of Carcinogenic Effects

It is not always simple to decide whether a chemical is a carcinogen or not, since available studies may be limited, inadequate or inconsistent. The EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

- Group A: Known Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal relationship between exposure to the chemical and human cancer.

- Group B: Probable Human Carcinogen. Adequate evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

- Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals with no reliable human data.

- Group D: Not classified. Inadequate evidence of carcinogenicity in animals.

- Group E: No evidence of carcinogenicity. No evidence of carcinogenicity in adequate tests in two different animal species or in both animal and epidemiology studies.

If toxicological evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimate excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually comes from lifetime exposure studies in animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction of noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is two liters of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure via ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any others. Because each model is based upon differing assumptions, the estimates which were derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose response data usually are available only for high levels of exposure, not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Non-Carcinogenic Effects

Absorption of chromium compounds from the gastrointestinal tract of animals and humans is low. Absorption of Cr(III) salts is only 0.5% to 3%, and absorption of Cr(VI) salts ranges from 2% to 10%. Organic chromium complexes may be absorbed more efficiently (10% to 25%).

1. Short-Term Exposure

Compounds of Cr(III) have low toxicity in man and animals. In rats, LD_{50} values for various Cr(III) salts range from 600 to 2,600 mg Cr(III)/kg (Smyth et al. 1969). The oral LD_{50} for Cr(VI) as $Na_2Cr_2O_7$ in rats is 19.8 mg Cr(VI)/kg (NIOSH 1983). Doses of 0.5 to 1.5 g of $K_2Cr_2O_7$ have been fatal in humans, and the estimated oral LDL_0 in children for $K_2Cr_2O_7$ is 26 mg/kg (9.2 mg Cr(VI)/kg) (NIOSH 1983).

Oral ingestion of lower quantities of chromium has few adverse health effects. Gross and Heller (1946) exposed young rats (number, strain and specific age were not provided) for two months to K_2CrO_4 in drinking water.

Adult mice were also evaluated, but the exposure period, as well as other experimental details, were not given. Therefore, only results obtained from the rat study are presented here. Rats (both male and female) were exposed to drinking water containing either 300 or 500 mg K_2CrO_4/L (80 mg Cr(VI)/L and 134 mg Cr(VI)/L, respectively). Using reported average body weights of 270 and 260 g, and assuming consumption of 28 mL water per day, the average ingested doses of Cr(VI) are calculated to be 8.3 and 14.4 mg/kg/day for groups receiving 80 and 134 mg Cr(VI)/L, respectively. After two months, the rats receiving 8.3 mg Cr(VI)/kg/day were described as normal. A "slight roughness of coat" was noted in rats receiving 14.4 mg Cr(VI)/kg/day, but this is not considered to be an adverse health effect, and this observation is not associated with other adverse health effects. Therefore, 14.4 mg Cr(VI)/kg/day represents the NOAEL in this study.

No studies are available to define the acute effects of Cr(III) in animals following oral exposure in drinking water. Studies in which Cr(III) was given to animals via the diet have not been considered, because the constituents of the diet significantly influence chromium absorption. For example, biologically complexed Cr(III) is absorbed much more readily than inorganic forms of chromium (Mertz 1976). While specific studies on the influence of food constituents on chromium absorption were not identified, there is an extensive amount of literature on the influence of food on the uptake of other metals (e.g., Rabar and Kostial 1981). Similarly, studies of the effects of chromium inhalation have not been considered, because absorption from the lungs may differ greatly from absorption from the GI tract. Additionally, pulmonary chromium does not appear to be in full equilibrium with chromium pools in the rest of the body (see Section III). Thus, the results from an inhalation study by Last et al. (1979) are immaterial for the development of HA values, because they deal only with localized effects of chromium on tracheal epithelium following inhalation exposure.

2. Long-Term Exposure

Schroeder et al. (1965) investigated the effects of 5 mg Cr(III)/L in drinking water on 46 male and 50 female Long Evans rats from the time of weaning until death. No significant effects of Cr(III) were observed in rats of either sex. Assuming an average weight of 330 g and an average daily water intake of 28 mL (Arrington 1972), the daily dose of Cr(III) was about 0.42 mg/kg/day. Since only one dose level of Cr(III) was studied, 0.42 mg/kg/day may be a low estimate of the NOAEL.

Ivankovic and Preussmann (1975) fed Cr(III) (as Cr_2O_3) to three groups of inbred BD rats (100 days old, 15 to 20 rats/group, including both male and female) five days per week for two years (600 feeding days). The diets fed to each group contained 1, 2 or 5% Cr_2O_3. The average cumulative doses of Cr_2O_3 were 0.6, 1.2 or 3.0 g/kg, corresponding to average daily doses of 293, 586 or 1,466 mg Cr(III)/kg/day for each group during the two year study. No significant differences (P value not given) in body weights were observed between the experimental groups and controls. Median survival times for all three dose groups were comparable with those of the controls. No macroscopic or histological findings were attributed to chromium treatment. The NOAEL from this study is 1,466 mg Cr(III)/kg/day. (The NOAEL calculated from this same study by EPA (1980) was 5,102 mg Cr_2O_3/kg/day, equivalent to 3,490 mg Cr(III)/kg/day.) However, this calculation appears to involve an inappropriate division by a factor of 0.35. Correcting for this error, a value of 1,786 mg Cr(III)/kg/day is obtained. This is similar to the ADI calculated above, with the difference being accounted for by assumed dietary intake and average weight values slightly different from those reported by the EPA (1980).

The effects of chronic Cr(VI) ingestion were studied by Anwar et al. (1961) in six-week-old female dogs (five dose groups with two animals per group) administered 0.45 to 11.2 mg Cr(VI)/L (as K_2CrO_4) in drinking water for four years. Assuming an average weight of 13 kg and an average daily water consumption of 350 mL (Arrington 1972), the average dose was between 0.012 and 0.30 mg Cr(VI)/kg/day. No abnormalities in physical condition, food consumption or growth rate were observed at any of the dose levels. The weights of the liver, kidney and spleen also remained normal at all dose levels. No significant changes (statistical analyses were not provided) were found in urinalyses for albumin, acetone, bile pigments, glucose and erythrocytes, or in blood analyses for erythrocyte count, total or differential leukocyte counts and hemoglobin. Chromium accumulation was observed in the liver, kidney and spleen, but appeared to be independent of the daily dose of chromium received by the dogs. The NOAEL from this study was 0.30 mg Cr(VI)/kg/day.

MacKenzie et al. (1958) studied the effects of chronic Cr(III) and Cr(VI) ingestion in rats. Both male and female Sprague Dawley rats (34 days old) were supplied with drinking water containing a series of doses up to 25 mg Cr/L, either as $CrCl_3$ (Cr(III)) or as K_2CrO_4 (Cr(VI)), for a period of one year. Assuming an average weight of 375 g for males and 290 g for females, and an average daily water intake of 28 mL (Arrington 1972), the average dose for males and females receiving 25 mg/L is calculated to be 1.87 and 2.41 mg Cr/kg/day, respectively. No significant adverse effects on appearance, weight gain, food consumption or blood chemistry were noted at any of the dose levels. However, the animals receiving the highest dose (25 mg/L) of Cr(VI) showed an approximate 20% reduction in water consumption.

Davids and Lieber (1951) have provided the only available study on human health effects of chromium in drinking water. For three years, a family of four persons on Long Island consumed drinking water from a private well that contained approximately 1 mg Cr(VI)/L (0.03 mg/kg/day for the 70 kg human). No adverse health effects were detectable in any of the family members as determined by physical examination (no further details were given).

B. Quantification of Non-Carcinogenic Effects

1. One-Day Health Advisory

There are no suitable data for calculating a Cr(III) or Cr (VI) One-day HA value for a 10-kg child. Therefore, the value calculated for the Ten-day

HA (below) will be taken as a conservative estimate of the appropriate One-day HA.

2. Ten-Day Health Advisory

There are no suitable data for calculating a Cr(III) Ten-day HA value for a 10-kg child.

The study by Gross and Heller (1946) has been selected to serve as the basis for calculation of a Cr(VI) Ten-day HA value for a 10-kg child. The NOAEL observed in this study was 134 mg Cr(VI)/L (14.4 mg/kg/day). It must be emphasized that these authors reported results with rats following two months of exposure. However, no short-term oral exposure studies are available to provide a more applicable basis for this calculation. Assumptions regarding absorption are not required in the Ten-day HA calculations, because the NOAEL is expressed in terms of an intake dose rather than an absorbed dose.

The Ten-day HA for the 10-kg child for Cr(VI) is calculated as follows:

$$\text{Ten-day HA} = \frac{(14.4\ \text{mg/kg/day})\ (10\ \text{kg})}{(1\ \text{L/day})\ (100)} = 1.4\ \text{mg/L}\ (1{,}400\ \mu\text{g/L})$$

where:

14.4 mg/kg/day = NOAEL following a 60-day oral exposure in rats (Gross and Heller 1946).

10 kg = assumed weight of child.

1 L/day = assumed water consumption by a 10-kg child.

100 = uncertainty factor. This uncertainty factor was selected based upon the NAS/ODW guidelines in which a NOAEL from an animal study is used.

3. Longer-Term Health Advisory

The study by MacKenzie et al. (1958) has been selected to serve as the basis for the Longer-term HAs. During this one year study in rats, both Cr(III) and Cr(VI) were administered in drinking water at a series of dose levels up to 25 mg/L (2.41 mg/kg/day for female rats) without significant adverse effects.

Using 2.41 mg/kg/day as the NOAEL, the Longer-term HA for the 10-kg child is calculated as follows:

$$\text{Longer-term HA} = \frac{(2.41 \text{ mg/kg/day})\ (10 \text{ kg})}{(1 \text{ L/day})\ (100)} = 0.24 \text{ mg/L } (240\ \mu\text{g/L})$$

where:

2.41 mg/kg/day = NOAEL, based on absence of effects on appearance, weight gain, food consumption or blood chemistry in rats exposed to Cr(III) or Cr(VI) via drinking water for one year.

10 kg = assumed weight of child.

100 = uncertainty factor. This uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from a study in animals is employed.

1 L/day = assumed water consumption by a 10-kg child.

Similarly, the Longer-term HA for a 70-kg adult, consuming 2 L/day of water, is calculated as follows:

$$\text{Longer-term HA} = \frac{(2.41 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})\ (100)} = 0.84 \text{ mg/L } (840\ \mu\text{g/L})$$

4. Reference Dose and Drinking Water Equivalent Level

The DWEL for chromium will be determined on the basis of the toxic effects of Cr(VI), and a separate DWEL will not be established for Cr(III) for the following reasons:

1. Cr(III) and Cr(VI) are in dynamic equilibrium, the degree of oxidation in an aqueous medium depending on factors such as pH, pO_2, presence of reducing agents, etc. In lake water (as opposed to sediments), Schroeder and Lee (1975) described the slow oxidation of Cr(III) to Cr(VI). The rate of this oxidation was accelerated by the addition of an oxidizing agent (25 mg/L MnO_2). Chlorination treatment of water (2 mg/L free residual chlorine) will effectively transform Cr(III) to Cr(VI). The normal presence of residual oxidizing capacity in treated water throughout the water

distribution system will assure maintenance of dissolved Cr in the oxidized state; therefore, drinking water at the point of consumption (i.e., the tap) is likely to contain Cr(VI).

2. Cr(VI) is more toxic than Cr(III), since only the hexavalent species readily crosses cell membranes. The nonmutagenicity of Cr(III), as contrasted with that of Cr(VI), can be directly attributed to this fact (Jennette 1979), even though Cr(VI) is reduced to Cr(III) intracellulary, and it is Cr(III) which reacts with DNA in the cells (Levis et al. 1977, 1978a). A standard based on Cr(VI), therefore, will be conservative with respect to any Cr(III) which might be present in water.

3. Even though orally ingested Cr(VI) is reduced in part during passage through the stomach, reduction is incomplete, and there is greater absorption (Donaldson and Barreras 1966) and greater tissue accumulation (MacKenzie et al. 1958) of chromium following ingestion of Cr(VI) than of Cr(III).

For the reasons stated, the DWEL is calculated for Cr(total), on the basis of the NOAEL observed for Cr(VI). Two studies were candidates to serve as the basis for this calculation. In a dog study, Anwar et al. (1961) observed a NOAEL of 11.2 mg Cr(VI)/L of drinking water, corresponding to 0.3 mg Cr(VI)/kg/day. Although this study was four years in duration, only two dogs per dose group were employed. This study was excluded on the basis of the small test group size. The study by MacKenzie et al. (1958), although it lasted only one year, employed more animals (16 rats/dose group). The NOAEL observed in this study was 2.41 mg Cr(VI)/kg/day. Due to increased sensitivity, the study by MacKenzie et al. (1958) is the most appropriate available study from which to derive the DWEL.

Assumptions regarding absorption are not required in the DWEL calculation, because the NOAEL is expressed in terms of an intake dose rather than an absorbed dose.

Using this study, the DWEL is derived as follows:

Step 1: Determination of RFD

$$\text{RfD} = \frac{(2.41 \text{ mg/kg/day})}{(100)\ (5)} = 0.0048 \text{ mg/L}$$

where:

2.41 mg/kg/day = NOAEL, based on absence of effects on appearance, weight gain, food consumption or blood chemistry in rats exposed to Cr(VI) via drinking water for one year (MacKenzie et al. 1958).

100 = uncertainty factor. This uncertainty factor was chosen in accordance with NAS/ODW guidelines in which a NOAEL from a study in animals is employed.

5 = additional uncertainty factor to compensate for less than lifetime exposure.

Step 2: Determination of DWEL

$$\text{DWEL} = \frac{(0.0048 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})} = 0.17 \text{ mg/kg/day } (170\ \mu g/L)$$

where:

0.0048 mg/kg/day = RfD

70 kg = assumed weight of adult.

2 L/day = assumed water consumption by 70-kg adult.

The Cr(VI) DWEL value may be compared with the current MCL of 0.05 mg total chromium/L recommended by EPA (1976). The calculated DWEL value is somewhat higher than the current MCL. The current MCL is apparently based on some of the same studies reported here (i.e., MacKenzie et al. 1958, Gross and Heller 1946) as well as other reports, but the means of selecting 0.05 mg/L appears arbitrary and is not well explained.

The calculated RfD value for Cr(VI) of 0.0048 mg/kg/day (equivalent to 0.34 mg/day for 70-kg adult) also compares favorably with the recommended daily adult intake level of 0.05 to 0.2 mg/day (50 to 200 µg/day) that is believed to be required for the protection of health (NAS 1980a, b).

C. Carcinogenic Effects

There is very little evidence to suggest that oral exposure to chromium can lead to cancer. Ivankovic and Preussmann (1975) exposed rats to 293, 586 and 1,466 mg Cr(III)/kg/day (administered as chromium oxide pigments) for two years, but observed no increase in the tumor rate over that of the control animals. Stocks (1957) attempted to correlate chromium concentrations in garden soil adjacent to different homes with the incidence of cancer in the corresponding household. No indication was given whether food grown in the soils analyzed was actually consumed. The level of significance of the slight correlations observed was not reported. In addition, the authors utilized median values instead of arithmetic means. The carcinogenicity of inhaled Cr(VI) is well established (Hayes et al. 1979), but the effects are observed primarily in the respiratory passages and in the lungs, and have no bearing on carcinogenic risk following oral exposure to the metal.

The genotoxic effects of chromium are well documented both in *in vivo* and *in vitro* studies. The pathway by which chromium exerts these effects (see Figure III-1) is believed to involve penetration of the cell membrane by Cr(VI), followed by intracellular reduction to Cr(III). Extracellular Cr(III) crosses the cell membrane, but less efficiently. Once formed inside the cell, Cr(III) can form tight complexes with DNA, accounting for its mutagenic potential. Compounds of both Cr(III) and Cr(VI) increase non-complementary nucleotide incorporation into DNA (Raffetto et al. 1977, Majone and Rensi 1979), with Cr(VI) being effective at lower doses. Exposure of cells to

Cr(VI) leads to increased cross-linking in DNA from rat liver and kidney. Therefore, chromium compounds may be mutagenic in humans. Petrilli and De Flora (1977, 1978b) reported positive Ames tests for Cr(VI); however, Cr(III) exerted no effect at relatively high concentrations (presumably because of its inability to penetrate cells). Similar results were reported by Gentile et al. (1981).

D. Quantification of Carcinogenic Effects

There is no evidence of carcinogenic effects following oral exposure to chromium. Thus, no assessments for carcinogenic risks from oral exposure to chromium have been performed. Inhalation of chromium, however, is associated with an increased frequency of lung cancer in humans. The International Agency for Research on Cancer (IARC) has performed an assessment of the degree of evidence for the carcinogenicity of chromium and certain specific chromium compounds in humans and experimental animals (WHO 1982). This assessment concluded that chromium and certain specific compounds are Group 1 chemicals (a causal relationship exists to demonstrate the chemicals are carcinogenic in humans). The limited relevance of this classification (which included consideration of available data from inhalation exposures) to the drinking water situation (oral exposure) should be noted.

E. Existing Guidelines and Standards

Recommended or established standards for chromium in the United States include: The ambient water quality criterion of 50 μg/L for Cr(VI) and 170 mg/L for Cr (III) (USEPA 1980); 1 μg/m^3 for carcinogenic forms of Cr(VI) in workplace air (NIOSH 1975); and 25 μg/m^3 TWA or 50 μg/m^3 ceiling for non-carcinogenic forms of Cr(VI) in workplace air (NIOSH 1975). Based on the study by Mancuso (1975), CAG has estimated the lifetime cancer risk due to a constant exposure to air containing 1 μg/m^3 of elemental chromium to be 1.2 x 10^{-2} (USEPA 1983).

An estimated adequate and safe intake for chromium of 50 to 200 μg/day for adults has been established (NAS 1980a, b). This range is based on the absence of signs of chromium deficiency in the major portion of the U.S. population, which consumes an average of 60 μg of chromium per day. Estimated daily adequate and safe intakes for younger age groups are derived by extrapolation on the basis of expected food intake as follows: 0.01 to 0.04 mg chromium for infants less than six months of age; 0.02 to 0.06 mg for infants between six and twelve months; 0.02 to 0.08 mg for children between the ages of one and three years; 0.03 to 0.20 mg for four- to six-year-old children; and 0.05 to 0.20 mg for children from seven years to adulthood. It should be noted that the calculated ADI value for Cr(VI) (0.34 mg/day) lies slightly above the range of levels (0.05 to 0.20 mg/day) estimated to be adequate to meet chromium dietary requirements.

F. Special Considerations

The following special considerations are identified for their potential impact on the risk assessment processes.

1. Risk Assessment Concerns

In general, chromium (VI) compounds are more readily absorbed and more readily penetrate into cells than Cr(III) compounds. As a result, Cr(VI) is the more toxic species. Because of the possible interconversion of Cr(III) to Cr(VI) in water, the DWEL for chromium is based on a NOAEL following oral ingestion of Cr(VI).

2. High Risk Populations

There is little evidence to suggest that there are special segments of the population that are at higher risk from the toxic effects of chromium than the general population. However, neonates generally retain greater fractional amounts of ingested metal compounds than do adults and may therefore be more susceptible to metal exposure (Kostial et al. 1983).

Patients with pernicious anemia and associated achlorhydria may also absorb more of an oral dose of Cr(VI), due to decreased reduction in the stomach to Cr(III) (Donaldson and Barreras 1966).

3. Interactions

Influences of chromium on vanadium toxicity have been reported. High dietary levels of chromium (500 µg/g of diet) were reported to increase the toxicity of vanadium (5 µg/g of diet) in chicks (Hunt and Nielsen 1979). However, high dietary levels of chromium (500 - 2,000 µg/g) were effective in overcoming growth depression and mortality in rats fed 20 µg vanadium/g of diet (Wright 1968 as reported in NAS 1980b). It is difficult to evaluate the significance and relevance of these conflicting results.

Purely a priori, it is likely that a diet high in reducing compounds would cause significant reduction of Cr(VI) to Cr(III) in the intestine; reduction of Cr(VI) to Cr(III) in the presence of gastric juice has been demonstrated (Donaldson and Barreras 1966). Such reduction, in turn, would diminish the absorption of chromium from the GI tract. Because the magnitude of this reduction has not been determined, no quantitative alteration of the HAs and DWEL can be made based upon this information.

4. Beneficial Effects

There is good evidence to suggest that chromium is an essential micronutrient. The presently recommended daily intake level by adults is 0.05 to 0.20 mg/day (NAS 1980a, b). The value calculated for the RfD (equivalent to 0.34 mg Cr(VI)/day for 70-kg adult) is somewhat above the recommended daily requirement. Thus, a slight margin of safety exists between required and potentially toxic concentrations of chromium.

G. Summary

The recommended values for the One-day, Ten-day and Longer-term HAs and the DWEL are summarized in Table VIII-1.

Table VIII-1 Summary of Toxicological Effects For Chromium

Value	Drinking Water Concentration, mg/L	Reference
One-Day HA for 10-kg Child	1.4[a]	Gross and Heller (1946)
Ten-Day HA for 10-kg Child	1.4	Gross and Heller (1946)
Longer-Term HA for 10-kg Child	0.24	MacKenzie et al. (1958)
Longer-Term HA for 70-kg Adult	0.84	MacKenzie et al. (1958)
DWEL (70-kg Adult)	0.17	MacKenzie et al. (1958)

[a] Ten-day HA was taken to be a conservative estimate of the One-day HA since inadequate short-term dose response data were found.

IX. REFERENCES

Altman PL, Dittmer DA. 1974. Biology data book, second edition. Volume III. Bethesda, MD: Fed. Am. Soc. Exp. Biol. 1447-1449.

Alexander J, Aseth J, Norseth T. 1982. Uptake of chromium by rat liver mitochondria. Toxicol. 24:115-122.

Anwar RA, Langham RF, Hoppert CA, Alfredson BV, Byerrum RU. 1961. Chronic toxicity studies: III. Chronic toxicity of cadmium and chromium in dogs. Arch. Environ. Health 3:456-460.

Arrington LR. 1972. The laboratory animals. In: Introductory laboratory animal science. The breeding, care and management of experimental animals. Danville, IL: Interstate Printers and Publishers, Inc. pp. 9-11.

Axelsson G, Rylander R. 1980. Environmental chromium dust and lung cancer mortality. Environ. Res. 23:469-476.

Axelsson G, Rylander R, Schmidt A. 1980. Mortality and incidence of tumors among ferrochromium workers. Br. J. Ind. Med. 37:121-127.

Baetjer AM. 1950. Pulmonary carcinoma in chromate workers. I. A review of the literature and report of cases. AMA Arch. Hyg. Occup. Med. 2:487-504.

Baetjer AM, Damron C, Budacz V. 1959a. The distribution and retention of chromium in men and animals. AMA Arch. Ind. Health 20:136-150.

Baetjer AM, Lowney JF, Steffee H, Budacz V. 1959b. Effect of chromium on incidence of lung tumors in mice and rats. AMA Arch. Ind. Health 20:124-135.

Bartlett RJ, Kimble JM. 1976a. Behavior of chromium in soils; I. Trivalent forms. J. Environ. Qual. 5:379-383.

Bartlett RJ, Kimble JM. 1976b. Behavior of chromium in soils; II. Hexavalent forms. J. Environ. Qual. 5:383-386.

Behari J, Chandra SV, Tandon SK. 1978. Comparative toxicity of trivalent and hexavalent chromium to rabbits. III. Biochemical and histological changes in testicular tissues. Acta Biol. Med. Ger. 37:463-468.

Bidstrup PL, Case RAM. 1956. Carcinoma of the lung in workmen in the bichromates-producing industry in Great Britain. Br. J. Ind. Med. 13:260-264.

Bigaliev AB, Elemesova MS. 1978. Influence of alpha-tocopherol on the cytogenetic effect of chromium. Tsitol. Genet. 12:414-416.

Bloomfield JJ, Blum W. 1928. Health hazards in chromium plating. Public Health Rep. 43:2330-2347.

Bovet P, Lob M, Grandjean M. 1977. Spirometric alterations in workers in the chromium electroplating industry. Int. Arch. Occup. Environ. Health 40:25-32.

Bowes JH, Kenten RH. 1948. The amino acid composition and titration curve of collagen. Biochem. J. 43:358-365.

Bowes JH, Kenten RH. 1949. The effect of deamination and esterification on the reactivity of collagen. Biochem. J. 44:142-152.

Burrows D. 1978. Chromium and the skin. Br. J. Dermatol. 99:587-595.

Cohen HA. 1966. Carrier specificity of tuberculin-type reaction to trivalent chromium. Arch. Dermatol. 93:34-40.

Cohen HA. 1976. The role of carrier in sensitivity to chromium and cobalt. Arch. Dermatol. 112:37-39.

Collins RJ, Fromm PO, Collings WD. 1961. Chromium excretion in the dog. Am. J. Physiol. 201:795-798.

Cotton FA, Wilkinson G. 1972. Advanced inorganic chemistry, 3rd ed. New York, NY: Interscience Publishers, pp. 830-845.

Czernielewski A, Brykalski D, Depczyk D. 1965. Environmental investigations on penetration of radioactive chromium (Cr^{51}) through the skin. Dermatologica 131:384-396.

Dalager NA, Mason TJ, Fraumeni JF, Hoover R, Payne WW. 1980. Cancer mortality among workers exposed to zinc chromate paints. J. Occup. Med. 22:25-29.

Davids HW, Lieber M. 1951. Underground water contamination by chromium wastes. Water Sewage Works 98:528-534.

Davidson IWF, Burt RL, Parker JC. 1974. Renal excretion of trace elements: Chromium and copper (38425). Proc. Soc. Exp. Biol. Med. 147:720-725.

Davies JM. 1978. Lung cancer mortality of workers making chrome pigments. Lancet 1:384.

Denton CR, Keenan RG, Birmingham DJ. 1954. The chromium content of cement and its significance in cement dermatitis. J. Invest. Dermatol. 23:189-192.

DiPaolo JA, Casto BC. 1979. Quantitative studies of *in vitro* morphological transformation of Syrian hamster cells by inorganic metal salts. Cancer Res. 39:1008-1013.

Donaldson RM Jr, Barreras RF. 1966. Intestinal absorption of trace quantities of chromium. J. Lab. Clin. Med. 68:484-493.

Eichhorn GL, Shin YA. 1968. Interaction of metal ions with polynucleotides and related compounds, XII: The relative effect of various metal ions on DNA helicity. J. Am. Chem. Soc. 90:7323-7328.

Eichhorn GL, Richardson C, Pitha J. 1971. Mispairing of nucleotide bases induced by metal ions. Proc. Am. Chem. Soc. 162nd Natl. Mtg. Washington, DC Abstract #17.

Enterline PE. 1974. Respiratory cancer among chromate workers. J. Occup. Med. 16:523-526.

Evan AP, Dail WG. 1974. The effects of sodium chromate on the proximal tubules of the rat kidney. Lab. Invest. 30:704-715.

Ewig RAG, Kohn KW. 1978. DNA-protein cross-linking and DNA interstrand cross-linking by haloethylnitrosoureas in L1210 cells. Cancer Res. 38:3197-3203.

Foulkes EC. 1974. Excretion and retention of cadmium, zinc and mercury by rabbit kidney. Am. J. Physiol. 227:1356-1360.

Fregert S, Hjorth N, Magnusson B, Bandmann HJ, Calnan CD, Cronin E, Malten K, Meneghini CL, Pirila V, Wilkinson DS. 1969. Epidemiology of contact dermatitis. Tran. St. John Hosp. Dermatol. Soc. 55:17-35.

Fregert S, Rorsman H. 1964. Allergy to trivalent chromium. Arch. Dermatol. 90:4-6.

Freund H, Atamian S. Fischer JE. 1979. Chromium deficiency during total parental nutrition. J. Am. Med. Assoc. 241:496-498.

Fristedt B, Lindqvist B, Schutz A. Ovrum P. 1965. Survival in a case of acute oral chromic acid poisoning with acute renal failure treated by haemodialysis. Acta Med. Scand. 177:153-159.

Fuwa K, Wacker WEC, Druyan R, Bartholomay AF, Vallee BL. 1960. Nucleic acids and metals, II. Transition metals as determinants of the conformation of ribonucleic acids. Proc. Natl. Acad. Sci. USA. 46:1298-1307.

Gentile JM, Hyde K, Schubert J. 1981. Chromium genotoxicity as influenced by complexation and rate effects. Toxicol. Lett. 7:439-448.

Gilani SH, Marano M. 1979. Chromium poisoning and chick embryogenesis. Environ. Res. 19:427-431.

Goldman M, Karotkin RH. 1935. Acute potassium bichromate poisoning. Am. J. Med. Sci. 189:400-403.

Goodenough U, ed. 1978. Mutation and repair, chapter 6. In: Genetics, second edition. New York: Holt, Rinehart and Winston. pp. 174-217.

Gray SJ, Sterling K. 1950. The tagging of red cells and plasma proteins with radioactive chromium. J. Clin. Invest. 29:1604-1613.

Griffin RA, Au AK, Frost RR. 1977. Effect of pH on adsorption of chromium from landfill-leachate by clay minerals. J. Environ. Sci. Health A 12:431-449.

Grogan CH, Oppenheimer H. 1955. Experimental studies in metal carcinogenesis. V. Interaction of Cr (III) and Cr (VI) compounds with proteins. Arch. Biochem. Biophys. 56:204-221.

Gross WG, Heller VG. 1946. Chromates in animal nutrition. J. Ind. Hyg. Toxicol. 28:52-56.

Gruber JE, Jennette KW. 1978. Metabolism of the carcinogen chromate by rat liver microsomes. Biochem. Biophys. Res. Commun. 82:700-706.

Gurson CT, Saner G. 1974. Effect of chromium on glucose utilization in marasmic protein calorie malnutrition. Am. J. Clin. Nutr. 24:1313-1319.

Gutierrez PL, Sarna T, Swartz HM. 1976. Experimental considerations in biological ESR studies. II. Chromium-tissue complexes detected by electron spin resonance. Phys. Med. Biol. 21:949-954.

Hartford WH. 1979. Chromium compounds. In: Grayson M, Eckroth D, eds. Kirk-Othmer encyclopedia of chemical technology, Vol. 6. New York, NY: John Wiley and Sons, pp. 82-120.

Hayes RB, Lilienfeld AM, Snell LM. 1979. Mortality in chromium chemical production workers: a prospective study. Int. J. Epidemiol. 8:365-374.

Hedenstedt A, Jenssen D, Lidesten BM, Ramel C, Rannug U, Stern RM. 1977. Mutagenicity of fume particles from stainless steel welding. Scan. J. Work Environ. Health 3:203-211.

Hem JD. 1970. Study and interpretation of the chemical characteristics of natural water, 2nd ed. U.S. Geological Survey Water-Supply Paper 1473. p. 199.

Herrmann H, Speck LB. 1954. Interaction of chromate with nucleic acids in tissues. Science 119:221.

Hopkins LL. 1965. Distribution in the rat of physiological amounts of injected Cr^{51} (III) with time. Am. J. Physiol. 209:731-735.

Hopkins LL, Schwarz K. 1964. Chromium (III) binding to serum proteins, specifically siderophilin. Biochem. Biophys. Acta 90:484-491.

Hueper WC, Payne WW. 1962. Experimental studies in metal carcinogenesis chromium, nickel, iron, arsenic. Arch. Environ. Health 5:445-462.

Huff JW, Sastry KS, Gordon MP, Wacker WEC. 1964. The action of metal ions on tobacco mosaic virus ribonucleic acid. Biochemistry 3:501-506.

Hunt C, Nielsen FH. 1979. The interaction between vanadium and chromium in the chick. Proc. Fed. Am. Soc. Exp. Biol. 38:449.

IARC. 1973. International Agency for Research on Cancer. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: Some inorganic and organometallic compounds, Vol. 2. Lyon, France: World Health Organization, pp. 100-125.

ICRP. 1975. International Commission for Radiological Protection. Physiological data for reference man. In: Report of the task group on reference man, No. 23. New York, NY: Permagon Press. pp. 335-365.

Iler RK. 1954. Process for the production of Werner type chromium complexes. U.S. Patent No. 2,683,156.

Ivankovic S, Preussman R. 1975. Absence of toxic and carcinogenic effects after administration of high doses of chromic oxide pigment in subacute and long term feeding experiments in rats. Food Cosmet. Toxicol. 13:347-351.

Jennette KW. 1979. Chromate metabolism in liver microsomes. Biol. Trace Elem. Res. 1:55-62.

Jennette KW. 1979. Microsomal reduction of the carcinogen chromate produces chromium (V). J. Am. Chem. Soc. 104:874-875.

Jennette KW. 1982. Microsomal reduction of the carcinogen chromate produces chromium (V). J. Am. Chem. Soc. 104:874-875.

Johansson A, Lundborg M, Hellstrom PA, Camner P, Keyser TR, Kirton SE, Natusch DFS. 1980. Effect of iron, cobalt and chromium dust on rabbit alveolar macrophages, a comparison with the effects of nickel dust. Environ. Res. 21:165-176.

Kanematsu N, Hara M, Kada T. 1980. REC assay and mutagenicity studies on metal compounds. Mutat. Res. 77:109-116.

Kaufman DB, DiNicola W. McIntosh R. 1970. Acute potassium dichromate poisoning. Am. J. Dis. Child. 119:374-376.

Kirkpatrick DC, Coffin DE. 1974. The trace metal content of representative Canadian diets in 1970 and 1971. J. Inst. Can. Sci. Technol. Aliment. 7:56-58.

Knudsen I. 1980. The mammalian spot test and its use for the testing of potential carcinogenicity of welding fume particles and hexavalent chromium. Acta Pharmacol. Toxicol. 47:66-70.

Kohn KW. 1979. DNA as a target in cancer chemotherapy: Measurement of macromolecular DNA damage produced in mammalian cells by anticancer agents and carcinogens. Methods Cancer Res. 16:291-345.

Kopp JF. 1969. The occurrence of trace elements in water. In: Hemphill D. ed. Trace substances in environmental health III. Columbia, MO: University of Missouri. pp. 59-73.

Kostial K, Simonovic I, Rabar I, Blanusa M, Landeka M. 1983. Age and intestinal retention of mercury and cadmium in rats. Environ. Res. 31:111-115.

Kraintz L, Talmage RV. 1952. Distribution of radioactivity following intravenous administration of trivalent chromium-51 in the rat and rabbit. Proc. Soc. Exp. Biol. Med. 81:490-492.

Kumpulainen JT, Wolf WR, Veillon C, Mertz W. 1979. Determination of chromium in selected United States diets. J. Agric. Food Chem. 27:490-494.

Lane BP, Mass MJ. 1977. Carcinogenicity and cocarcinogenicity of chromium carbonyl in heterotrophic tracheal grafts. Cancer Res. 37:1476-1479.

Langard S. 1980. A survey of respiratory symptoms and lung function in ferrochromium and ferrosilicon workers. Int. Arch. Occup. Environ. Health 46:1-9.

Langard S, Andersen A, Gylseth B. 1980. Incidence of cancer among ferrochromium and ferrosilicon workers. Br. J. Ind. Med. 37:114-120.

Langard S. Norseth T. 1975. A cohort study of bronchial carcinomas in workers producing chromate pigments. Br. J. Ind. Med. 32:62-65.

Laskin S, Kuschner M, Drew, RT. 1970. Studies in pulmonary carcinogenesis. In: Hanna MG, Nettesheim P, Gilber JR, eds. Inhalation carcinogenesis. Washington, DC: U.S. Atomic Energy Commission. pp. 321-351.

Last JA, Raabe OG, Moore PF, Tarkington BK. 1979. Chromate inhibition of metabolism by rat tracheal explants II. In-vivo exposures. Toxicol. Appl. Pharmacol. 47:313-322.

Latimer WM. 1952. Oxidation potentials, 2nd ed. New York, NY: Prentice-Hall, pp. 245-250.

Levine RA, Streeten DHP, Doisy RJ. 1968. Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects. Metabolism 17:114-125.

Levis AG, Majone F. 1979. Cytotoxic and clastogenic effects of soluble chromium compounds on mammalian cell cultures. Br. J. Cancer 40:523-533.

Levis AG, Buttignol M, Vettorato L. 1977. Inhibition of DNA synthesis in BHK fibroblasts treated in vitro with potassium dichromate. Experientia 33:82-84.

Levis AG, Bianchi V, Tamino G, Pegoraro B. 1978a. Cytotoxic effects of hexavalent and trivalent chromium on mammalian cells in vitro. Br. J. Cancer 37:386-396.

Levis AG, Buttignol M, Bianchi V. Sponza G. 1978b. Effects of potassium dichromate on nucleic acid and protein syntheses and on precusor uptake in BHK fibroblasts. Cancer Res. 38:110-116.

Lim TH, Sargent T, Kusuhov N. 1983. The kinetics of the trace element chromium (III) in the human body. Am. J. Physiol. 244:R445-R454.

Lofroth G. 1978. The mutagenicity of hexavalent chromium is decreased by microsomal metabolism. Naturwissenschaften 65:207-208.

Love CH. 1947. German production of some of the more important inorganic pigments. Washington, DC: Hobart Publishing Co. pp. 47-63.

Machle W. Gregorius F. 1948. Cancer of the respiratory system in the United States chromate-producing industry. Public Health Rep. 63:1114-1127.

MacKenzie RD, Byerrum RU, Decker CF, Hoppert CA, Langham RF. 1958. Chronic toxicity studies. II. Hexavalent and trivalent chromium administered in drinking water to rats. AMA Arch. Ind. Health 18:232-234.

Majone F, Levis AG. 1979. Chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells treated in vitro with hexavalent chromium compounds. Mutat. Res. 67:231-238.

Majone F, Rensi D. 1979. Mitotic alterations, chromosome aberrations and sister chromatid exchanges induced by hexavalent and trivalent chromium on mammalian cells in vitro. Caryologia 32:379-392.

Mali JWH, Malten K, Van Neer FCJ. 1966. Allergy to chromium. Arch. Dermatol. 93:41-44.

Mancuso TF. 1951. Occupational cancer and other health hazards in a chromate plant: a medical appraisal: II. Clinical and toxicologic aspects. Ind. Med. Sug. 20:393-407.

Mancuso TF. 1975. Internation conference on heavy metals in the environment. Toronto, Ontario, Canada. Oct. 27-31. (Cited in Towill et al. 1978).

Mancuso TF, Hueper WC. 1951. Occupational cancer and other health hazards in a chromate plant: a medical appraisal. I. Lung cancers in chromate workers. Ind. Med. Surg. 20:358-363.

Marino DF, Ingle JD Jr. 1981. Determination of chromium VI in water by lophine chemiluminescence. Anal. Chem. 53:294-298.

Mass MJ, Lane BP. 1976. Effect of chromates on ciliated cells of rat tracheal epithelium. Arch. Environ. Health 31:96-100.

Mathur AK, Chandra SV, Tandon SK. 1977. Comparative toxicity of trivalent and hexavalent chromium to rabbits. II. Morphological changes in some organs. Toxicology 8:53-61.

Meranger JC, Smith DC. 1972. The heavy metal content of a typical Canadian diet. Can. J. Public Health 63:53-57.

Mertz W. 1969. Chromium occurrence and function in biological systems. Physiol. Rev. 49:163-239.

Mertz W. 1974. The newer essential trace elements, chromium, tin, vanadium, nickel and silicon. Proc. Nutr. Soc. 33:307-313.

Mertz W. 1976. Chromium and its relation to carbohydrate metabolism. In: Burch RE, Sullivan JF, eds. Symposium on trace elements. Med. Clin. North Am. 60:739-744.

Mertz W, Anderson RA, Wolf WR, Roginski EE. 1978. Progress in chromium nutrition research. In: Kirchgessner M, ed. Trace element metabolism in man and animals. Proc. Third Int. Symp. Freising July 1977, pp. 272-278.

Mertz W, Roginski EE, 1971. Chromium metabolism: the glucose tolerance factor. In: Mertz W. Cornatzer WE, eds. Newer trace elements in nutrition. New York, NY: Marcel Dekker, pp. 123-151.

Mertz W, Roginski EE, Feldman FJ, Thurman DE. 1969. Dependence of chromium transfer into the rat embryo on the chemical form. J. Nutr. 99:363-367.

Mertz W, Roginski EE, Reba RC. 1965. Biological activity and fate of trace quantities of intravenous chromium (III) in the rat. Am. J. Physiol. 109:489-494.

Michael SE. 1939. The precipitation of proteins with complex salts. Biochem. J. 33:924-930.

Milby TH, Key MM, Gibson RL, Stokinger HE. 1964. Chemical hazards. In: Gafafer WM, ed. Occupational diseases: A guide to their recognition. Washington, DC: Public Health Service Publ. 1097, pp. 63-67, 120-122.

Moeschlin S. 1965. Poisoning: diagnosis and treatment, 1st Am. Ed. New York, NY: Grune and Stratton, pp. 145-149.

Morning JL. 1980. Chromium. In: Mineral commodities summary. Washington, DC: U.S. Bureau of Mines, pp. 34-35.

Murray MJ, Flessel CP. 1976. Metal polynucleotide interactions: a comparison of carcinogenic and non-carcinogenic metals in vitro. Biochem. Biophys. Acta 425:256-261.

Naismith WEF. 1958. The cross-linking of conarachin II with metal salts. Arch. Biochem. Biophys. 73:255-261.

Nakamuro K, Yoshikawa K, Sayato Y, Kurata H. 1978. Comparative studies of chromosomal aberration and mutagenicity of trivalent and hexavalent chromium. Mutat. Res. 58:175-181.

NAS. 1974. National Academy of Sciences. Water quality criteria 1972. Washington, DC: National Academy Press, p. 62.

NAS. 1980a. National Academy of Sciences. Recommended dietary allowances, 9th rev. ed. Washington, DC: National Academy Press, pp. 159-161.

NAS. 1980b. National Academy of Sciences. Drinking water and health, Vol. 3, Washington, DC: National Academy Press, pp. 266, 364-369, 374-375.

Nath R, Minocha J. Lyall V, Sunder S, Kumar V, Kapoor S, Dhar KL. 1979. Assessment of chromium metabolism in maturity onset and juvenile diabetes using chromium-51 and therapeutic response of chromium administration on plasma lipids, glucose tolerance and insulin levels. In: Snapcott D, Hubert J, eds. Chromium in nutrition and metabolism. Elsevier/North Holland Biomedical Press, pp. 213-221.

Nestmann ER, Matula TI, Douglas GR, Bora KC, Kowbel DJ. 1979. Detection of the mutagenic activity of lead chromate using a battery of microbial tests. Mutat. Res. 66:357-365.

Nettesheim P, Hanna MG. Doherty DG, Newell RF, Hellman A. 1970. Effects of chronic exposure to air pollutants on the respiratory tracts of mice: histopathological findings. In: Nettesheim and Deatherage, eds. Morphology of experimental respiratory carcinogenesis. Washington, DC: U.S. Atomic Energy Commission, pp. 437-448.

Newbold RF, Amos J, Connell JR. 1979. The cytotoxic, mutagenic and clastogenic effect of chromium-containing compounds on mammalian cells in culture. Mutat. Res. 67:55-63.

Nicholson TF, Shepherd GW. 1959. The effect of damage to various parts of the renal tubule on the excretion of phosphate by the dog's kidney. Can. J. Biochem. Physiol. 37:103-111.

NIOSH. 1975. National Institute for Occupational Safety and Health. Occupational exposure to chromium VI. Criteria document HEW(NIOSH) 76-129.

NIOSH. 1983. Registry of Toxic Effects of Chemical Substances (RTECS). Vol. 2. Cincinnati, OH: National Institute of Occupational Safety and Health.

Nishimura M, Umeda M. 1978. Mutagenic effect of some metal compounds on cultured mammalian cells. Mutat. Res. 54:246-247.

Nomiyama H, Yotoriyama M, Nomiyama K. 1980. Normal chromium levels in urine and blood of Japanese subjects determined by direct flameless atomic absorption spectrophotometry, and valency of chromium in urine after exposure to hexavalent chromium. Am. Ind. Hyg. Assoc. 41:98-102.

NRC. 1974. National Research Council. Chromium. Washington, DC: National Academy of Sciences.

Partington CN. 1950. Acute poisoning with potassium bichromate. Br. Med. J. 2:1097-1098.

Payne WW. 1960. Production of cancers in mice and rats by chromium compounds. AMA Arch. Ind. Health 21:530-535.

Pehelin VA, Grigor'eva NV, Izmailova VN. 1963. Fixation of polypeptide chains in two conformations. Dokl. Akad. Nauk SSSR 151:134-135. Reviewed in Chem. Abstr. 59:10229g.

Petrilli FL, De Flora S. 1977. Toxicity and mutagenicity of hexavalent chromium on Salmonella typhimurium. Appl. Environ. Microbiol. 33:805-809.

Petrilli FL, De Flora S. 1978a. Oxidation of inactive trivalent chromium to the mutagenic hexavalent form. Mutat. Res. 58:167-173.

Rabar I, Kostial K. 1981. Bioavailability of cadmium in rats fed various diets. Arch. Toxicol. 47:63-66.

Raffetto G, Parodi S, Parodi C, DeFarrari M, Troiano R, Brambilla G. 1977. Direct interaction with cellular targets as the mechanism for chromium carcinogenesis. Tumori 63:503-512.

Ridgway LP, Karnofsky DA. 1952. Effects of metals on the chick embryo: toxicity and production of abnormalities in development. Ann. N.Y. Acad. Sci. 55:203-215.

Robertson FN. 1975. Hexavalent chromium in the ground water in Paradise Valley, Arizona. Ground Water 13:516-527.

Roe FJC, Carter RL. 1969. Chromium carcinogenesis: calcium chromate as a potent carcinogen for the subcutaneous tissues of the rat. Br. J. Cancer 13:172-176.

Rollinson CL, Rosenbloom EW. 1969. Coordination in biochemistry: reactions of chromium (III). In: Kirschner, ed. Coordination chemistry. New York, NY: Plenum Press. pp. 108-125.

Royle H. 1975a. Toxicity of chromic acid in the chromium plating industry (1). Environ. Res. 10:39-53.

Royle H. 1975b. Toxicity of chromic acid in the chromium plating industry (2). Environ. Res. 10:141-163.

Samitz MH, Katz S. 1963. Preliminary studies on the reduction and binding of chromium with skin. Arch. Dermatol. 88:816-819.

Samitz MH, Katz S. 1964. A study of the chemical reactions between chromium and skin. J. Invest. Dermatol. 43:35-43.

Saner G. 1979. Chromium and glucose metabolism in children. In: Snapcott D, Hubert J, eds. Chromium in nutrition and metabolism. Elsevier/North Holland Biomedical Press, pp. 129-144.

Saner G. 1980. Biochemistry and physiologic role of chromium. In: Chromium in nutrition and disease. New York, NY: Alan R. Liss, Inc. pp. 7-21.

Saner G, Gurson CT. 1976. Hair chromium concentration in newborns and their mothers. Nutr. Rep. Int. 14:155-164.

Sarto F, Cominato I, Bianchi V, Levis AG. 1982. Increased incidence of chromosomal aberrations and sister chromatid exchanges in workers exposed to chromic acid (CrO_3) in electroplating factories. Carcinogenesis 3:1011-1016.

Schroeder HA, Balassa JJ. 1965. Influence of chromium, cadmium and lead on rat aortic lipids and circulating cholesterol. Am. J. Physiol. 209:433-437.

Schroeder HA, Balassa JJ, Tipton IH. 1962. Abnormal trace metals in man - chromium. J. Chron. Dis. 15:941-964.

Schroeder HA. 1970. Chromium. American Petroleum Institute Air Quality Monograph No. 70-15. Washington, DC.

Schroeder HA, Balassa JJ. 1965. Influence of chromium, cadmium and lead on rat aortic lipids and circulating cholesterol. Am. J. Physiol. 209:433-437.

Schroeder HA, Balassa JJ, Tipton IH. 1962. Abnormal trace metals in man - chromium. J. Chron. Dis. 15:941-964.

Schroeder HA, Balassa JJ, Vinton WH Jr. 1965. Chromium, cadmium and lead in rats: effects on life span, tumors and tissue levels. J. Nutr. 86:51-66.

Schroeder DC, Lee GF. 1975. Potential transformations of chromium in natural waters. Water Air Soil Pollut. 4:355-365.

Sharma BK, Singhal PC, Chugh KS. 1978. Intravascular haemolysis and acute renal failure following potassium dichromate poisoning. Postgrad. Med. J. 54:414-415.

Shin YA, Heim JM, Eichhorn GL. 1972. Interaction of metal ions with polynucleotides and related compounds. XX. Control of the conformation of polyriboadenylic acid by divalent metal ions. Bioinorg. Chem. 1:149-163.

Sirover MA, Loeb LA. 1976. Infidelity of DNA synthesis in vitro: screening for potential metal mutagens or carcinogens. Science 194:1434-1436.

Slater JP, Tamir I, Loeb LA, Mildvan AS. 1972. The mechanism of *Escherichia coli* deoxyribonucleic acid polymerase I. J. Biol. Chem. 247:6784-6794.

Smyth HF, Carpenter CP, Weil CS, Pozzani UC, Striegel JA, Nycum JS. 1969. Range finding toxicity data: List VII. Am. Ind. Hyg. Assoc. Journal 30:470.

Spruit D, Van Neer FCJ. 1966. Penetration rate of Cr (III) and Cr (VI). Dermatologica 132:179-182.

Stacy BD, Thorburn GD. 1966. Chromium-51 ethylenediamine tetraacetate for estimation of glomerular filtration rate. Science 152:1076-1077.

Stanforth, R, Ham R, Anderson M, Stegman R. 1979. Development of a synthetic municipal landfill leachate. J. Water Pollut. Control Fed. 51:1965-1975.

Steffee CH, Baetjer AM. 1965. Histopathologic effects of chromate chemicals. Arch. Environ. Health 11:66-75.

Stella M, Montaldi A, Rossi R, Rossi G, Levis AG. 1982. Clastogenic effects of chromium on human lymphocytes in vitro and in vivo. Mutation Res. 101:151-164.

Stocks P. 1957. Cancer incidence in North Wales and Liverpool region in relation to habits and environment. B.E.C.C. 35th Annual Report, Supplement to Part II, pp. 95-127.

Sunderman FW Jr. 1971. Metal carcinogenesis in experimental animals. Food Cosmet. Toxicol. 9:105-120.

Taylor FH. 1966. The relationship of mortality and duration of employment as reflected by a cohort of chromate workers. Am. J. Public Health 56:218-229.

Tkeshelashvili LK, Shearman CW, Koplitz RM, Zakour RA, Loeb LA. 1979. Fidelity of DNA synthesis: effects of As, Se, and Cr. Cancer Res. 20:263.

Tkeshelashvili LK, Shearman CW, Zakour RA, Koplitz RM, Loeb LA. 1980. Effects of arsenic, selenium, and chromium on the fidelity of DNA systhesis. Cancer Res. 40:2455-2460.

Toepfer EW, Mertz W, Polansky MM, Roginski EE, Wolf WR. 1977. Preparation of chromium containing material of glucose tolerance factor activity from brewer's yeast extract and by synthesis. J. Agric. Food Chem. 25:162-166.

Tola S, Hernberg S, Collan Y, Linderborg H, Korkala ML. 1980. A case control study of the etiology of nasal cancer in Finland. Int. Arch. Occup. Environ. Health 46:79-85.

Trama FB, Benoit RJ. 1960. Stream pollution: toxicity of hexavalent chromium to bluegills. J. Water Pollut. Control Fed. 32:868-877.

Tsapakos MJ, Hampton TH, Jennette KW. 1981. The carcinogen chromate induces DNA crosslinks in rat liver and kidney. J. Biol. Chem. 256:3623-3626.

Tsuda H, Kato K. 1976. Potassium dichromate-induced chromosome aberrations and its control with sodium sulfite in hamster embryonic cells in vitro. Gann 67:469-470.

Ulmer NS. 1986. Effect of chlorine on chromium speciation in tap water. United States Environmental Protection Agency. Water Engineering Research Laboratory. EPA/600/M-86/015 June.

USEPA. 1976. U.S. Environmental Protection Agency. National interim primary drinking water regulations. EPA 570/9-76-003. Washington, DC: U.S. Environmental Protection Agency, pp. 63-64.

USEPA. 1980. U.S. Environmental Protection Agency. Ambient water quality criteria for chromium. EPA 440/5-80-035. Washington, DC: U.S. Environmental Protection Agency.

USEPA. 1983. U.S. Environmental Protection Agency. Health assessment document for chromium. Review Draft. EPA 600/8-82-014A. Research Triangle Park, NC: U.S. Environmental Protection Agency.

USEPA. 1984. U.S. Environmental Protection Agency. Environmental Monitoring Systems Laboratory. Computer printout: frequency distributions by site/year for chromium, the results of samples collected at National Air Surveillance Network sites. Unpublished.

USPHS. 1953. U.S. Public Health Service. Health of workers in chromate producing industry. U.S. Public Health Service Publication No. 192. Washinqton, DC: U.S. Government Printing Office, 131 pp.

USPHS. 1962. U.S. Public Health Service. Drinking water standards. U.S. Public Health Service Publication No. 956. Washington, DC: U.S. Government Printing Office, pp 36-39.

USPHS. 1976. U.S. Public Health Service. National Center for Health Statistics (NCHS) Growth Charts, 1976. (HRA) 76-1120 Monthly Vital Statistics Report 25(3), 22 pp.

Van Neer FCJ. 1965. Reacties op intracutane injectie van drieen zeswaardige chroomverbindingen bij gesensibiliseerde mensen, varkens en cavia's. Ned. Tijdschr. Geneesk. 189:1684.

Venitt S, Levy LS. 1974. Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis. Nature 250:493-495.

Visek WJ, Whitney IB, Kuhn USG III, Comar CL. 1953. Metabolism of ^{51}Cr by animals as influenced by chemical state (20729). Proc. Soc. Exp. Biol. Med. 84:610-615.

Vittorio PV, Wight EW, Sinnott BE. 1962. The distribution of chromium-51 in mice after intraperitoneal injection. Can. J. Biochem. Physiol. 40:1677-1683.

Wacker WEC, Vallee BL. 1959a. Chromium, manganese, nickel and other metals in RNA. Fed. Proc. Fed. Am. Soc. Exp. Biol. 18:345.

Wacker WEC, Vallee BL. 1959b. Nucleic acids and metals. I. Chromium, manganese, nickel, iron and other metals in ribonucleic acid from diverse biological sources. J. Biol. Chem. 234:3257-3262.

Warren G, Schultz P, Bancroft D, Bennett K, Abbott EH, Rogers S. 1981. Mutagenicity of a series of hexacoordinate chromium (III) compounds. Mutation Res. 90:111-118.

Weast RC, ed. 1971. Handbook of chemistry and physics, 52nd edition. Cleveland, OH: CRC Press, pp. B-65, B-83, B-84, B-122, B-137.

Weinstein IB. 1978. Current concepts on mechanisms of chemical carcinogenesis. Bull. N.Y. Acad. Med. 54:366-383.

Westbrook JH. 1979. Chromium and chromium alloys. In: Grayson M, Eckroth D, eds., Kirk-Othmer encyclopedia of chemical technology, Vol. 6. New York, NY: John Wiley and Sons, pp. 54-82.

Wild D. 1978. Cytogenetic effects in the mouse of 17 chemical mutagens and carcinogens evaluated by the micronucleus test. Mutat. Res. 56:319-327.

Windholz M, ed. 1976. The Merck Index: An encyclopedia of chemicals and drugs, 9th ed. Rahway, NJ: Merck and Co., Inc. pp. 228-289.

WHO. 1982. World Health Organization. IARC monographs on the evaluation of carcinogenic risk to humans. Chemicals, industry processes and industries associated with cancer in humans. International Agency for Research on Cancer Monographs Vol. 1 to 29. Suppl. 4. Geneva: World Health Organization.

Wild D. 1978. Cytogenetic effects in the mouse of 17 chemical mutagens and carcinogens evaluated by the micronucleus test. Mutat. Res. 56:319-327.

Windholz M, ed. 1976. The Merck Index: An encyclopedia of chemicals and drugs, 9th ed. Rahway, NJ: Merck and Co., Inc. pp. 228-289.

Yamamoto A, Wada O, Ono T. 1981. Low-molecular-weight, chromium-binding substance in mammals. Toxicol. Appl. Pharmacol. 59:515-523.

Zakour RA, Tkeshelashvili LK, Shearman CW, Koplitz RM, Loeb LA. 1981. Metal-induced infidelity of DNA synthesis. J. Cancer Res. Clin. Oncol. 99:187-196.